A STUDY ON THE MICROBIOLOGICAL PROFILE OF BLOOD STREAM INFECTIONS IN PATIENTS ADMITTED IN INTENSIVE CARE UNIT IN A TERTIARY CARE HOSPITAL

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CERTIFICATE

This is to certify that this dissertation titled "**A STUDY ON MICROBIOLOGICAL PROFILE OF BLOOD STREAM INFECTIONS IN PATIENTS ADMITTED IN INTENSIVE CARE UNIT IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **DR.N.DEEPA,** during the period of her Post graduate study from 2009 to 2013 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-600003, in partial fulfillment of the requirement for M.D. MICROBIOLOGY degree Examination of The Tamilnadu Dr.M.G.R. Medical University to be held in April 2013.

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DECLARATION

I declare that the dissertation entitled "**A STUDY ON MICROBIOLOGICAL PROFILE OF BLOOD STREAM INFECTIONS IN PATIENTS ADMITTED IN INTENSIVE CARE UNIT IN A TERTIARY CARE HOSPITAL**" is submitted by me for the degree of M.D. is the record work carried out by me during the period of October 2010 to September 2012 under the guidance of **Prof.Dr.S.THASNEEM BANU, M.D.** Professor of Microbiology, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Microbiology (Branch IV) examination to be held in April 2013.

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INTRODUCTION

Blood stream infections (BSI) are the major cause of morbidity & mortality among patients admitted in Intensive care unit & surveillance of etiological agents in these infections are important for their prevention & treatment. Blood stream infection is the infection that required one or more cultures positive for a bacteria or a fungus of blood samples obtained in the presence of fever(>38^0C) not attributable to other causes(based on US centers of Disease control & prevention[1]).

Community acquired Bacteremia (CAP) was defined if the first positive blood culture was obtained before or within 48 hours of hospitalization. Blood stream infections are considered to be nosocomial if signs & symptoms of these infections became evident after 48 hours following hospital admission and/or if the patient had been hospitalized during the 2 weeks before the current admission.

The invasion of microorganisms in the circulating blood pose amajor threat to every organ in the body leading to serious consequences including shock, multiple Organ failure, DIC & Death. Blood stream infections with primary diseases admitted in ICU are Infective Endocarditis, CAP(community acquired pneumonia), Uro-sepsis & Meningitis[2]. BSI with Secondary Bacteremia are infections resulting from health care

interventions such as Vascular catheter insertion, infection following Urinary catheter related sepsis, infection of Surgical sites & infection arising out of hospital acquiredor ventilator associated pneumonia.Vascular access devices are inserted in critically ill patients for the maintenance of fluid Electrolyte Balance, administration of Drugs, maintaining nutritional requirements, monitoring vital parameters, promoting vital organ support & doing essential investigation[3].

The only way to avoid infections from this intervention is strict attention to asepsis during insertion of vascular access devices, regular review of each vascular channel so that they are kept as long as essential. The use of Chlorhexidine-based preparations & insertion of Central line through the Subclavian access reduce infection rate[4,5] .The patients with BSI manifest clinically with systemic signs of infection such as Fever, Leucocytosis, raised inflammatory markers. Blood cultures obtained from both peripheral &vascular access device are to be taken within 15 minute[6] detects CR-BSI .So the patients in ICU with sepsis are empirically treated with Glycopeptide antibiotics like Vancomycin to cover Gram positive pathogens(Methicillin sensitive and methicillin resistant *Staphylococcus aureus and S.epidermidis*). In many cases of CRBSI removal of vascular access device is the mainstay of treatment to prevent further complication[2,3].

According to one American study, the incidence of Bacteremia in critical care settings was estimated to be 3 cases per 1000 population[7]with Mortality rate between 20 % to 50 % & Mean mortality rate of 28.6%. Similar study showed Mortality rate of 34% at 28 days & 45% at 5 months. Patients who developed Sepsis after the 2nd day in the hospital had even higher mortality than those who were septic on admission[5].

The most common bacteria isolated from patients in ICU are gram positive aerobic bacteria (*S.aureus, Enterococcus*) and gram negative aerobic bacteria (*Enterobacteriaceae, Pseudomonas aeruginosa*) & the common fungi include *Candida albicans* in both immune competent & immune compromised patients[2]. *CONS* which was previously considered as contaminants have increased in clinical importance & are now recognized as pathogens[4,9]. They are the etiologic agents of catheter associated bacteremia in patients with Vascular & other prosthesis.So judging its clinical significance is very challenging.

Blood cultures arethe most important laboratory test performed in the diagnosis of serious infection and leads to a definitive diagnosis against the causative organisms.So the Blood culture is considered as the gold standard for the detection of bacteremia[4]. This study was taken in our institution to evaluate the prevalence of Septicaemia in ICU patients in our setup in relation to their source of infection. It is done to ascertain the importance of

Blood culture examination for the detection of BSI in ICU patients that helps in treating and decreasing is the morbidity & mortality due to Community acquired & Nosocomial Blood stream infections.There is considerable increase in incidence of vascular infection caused by bacteria that are normally considered avirulent. So it is important to distinguish between contaminants & pathogen.[13]

TO BE A PROBABLE CONTAMINANT[2]:

- Growth of *CONS*, *bacillus* spp., *Corynebacterium* spp., in only one of several cultures.
- Growth of multiple organisms from only one of several cultures.
- The clinical signs are not consistent with sepsis.
- The organism causing the infection at the primary site & the organism isolated from the blood culture are different.

TO BE A PROBABLE PATHOGEN[2,7]:

- Growth of same organisms in repeated cultures obtained either at different times or from different anatomical sites.
- Growth of organisms from suspected case of Endocarditis.

- Growth of organisms from members of *Enterobacteriaceae*, S *pneumonia, Gram negative anaerobes & S Pyogenes.*
- Isolation of commensal bacteria from Blood culture of patients suspected to be Bacteremic (Immunocompromised patients or those valves).

AIMS & OBJECTIVES

1. To isolate the pathogen causing infection in intensive care unit.
2. To identify the possible source of infection.
3. To determine the antimicrobial susceptibility pattern of the isolates.
4. To study the resistance pattern of the common isolates.

REVIEW OF LITERATURE

HISTORY

The term Sepsis was derived from a Greek word meaning **"PUTRID[3]"**. It was believed that putrefaction of wound was caused by contact with air & leading to death when theprocess of putrefaction reached the blood (Septicaemia). In the 19th century, the concept of infection as a cause of sepsis was introduced by Austrian obstetrician, Ignaz Philip Semmelweis & the English surgeon Joseph Lister.Since then the term Sepsis was closely related to Bacterial infection.

DEFINITION

The most widely used set of definitions was developed by consensus committee of experts in 1992[15,16]. The American College of chest physicians / society of critical care medicine (ACCP/ SCCM) consensus conference defined Sepsis as a systemic inflammatory response syndrome (SIRS) caused by infectious process[2].

SIRS (SYSTEMIC INFLAMMATORY RESPONSE SYNDROME)[15]:

SIRS is an abnormal generalized inflammatory reaction in organs remote from the initial insult. SIRS is defined as the systemic response to a wide range of stresses. Currently used Criteria include $\geq$2 of the following.

SIRS CRITERIA[3,7,15,16]:

- Temperature > 38ºC or < 36ºC
- Heart Rate > 90 beats / minute
- Respiratory rate > 20 breaths / min
- WBC > 12000 Cells / mm^3 or < 4000 Cells / mm^3 or > 10% immature neutrophils (band) forms.

SEPSIS :

Sepsis is defined as an invasion of microorganisms or their toxins into the blood stream together with the host response to this invasion.

If SIRS occurs in a patient with proven or suspected infection, it is known as sepsis. SIRS is called sepsis in the American consensus scheme[16].

BACTEREMIA:

Presence of bacteria in blood, as evidenced by positive blood cultures.

SEPTICAEMIA:

Presence of microbes or their toxins in blood. Septicaemia is a clinical syndrome characterized by fever, chills, malaise, tachycardia, hyperventilation and toxicity (or) prostration[19].

SEVERE SEPSIS :

Sepsis with one or more signs of organ dysfunction of the following.[16,17]

1. CVS – SBP $\leq$ 90 mm Hg
2. Renal – Urine output < 0.5ml/kg/hr for 1 hr
3. Respiratory – PO_2 / FIO_2 < 250
4. Hematologic – platelet count < 80,000/ml or 50% decrease in platelet count recorded for past 3 days

SEPTIC SHOCK[15]:

Sepsis with hypotension (SBP<90 mmHg, for at least one hour despite adequate fluid resuscitation.

RETRACTORY SEPTIC SHOCK[15,17]:

Septic shock that lasts for > 1 hour & does not respond to fluid or vasopressor administration.

MULTIPLE ORGAN DYSFUNCTION SYNDROME (MODS)[15]:

Dysfunction of more than one organ, requiring intervention to maintain homeostasis.

Another American conference in 2001, reconsidered definitions for Sepsis and proposed a new system for staging sepsis based on

predisposition insult,infection response and organ dysfunction (known as PIRO)[3]. For simplicity and convenience, the 1992 definitions are used widely.

EPIDEMIOLOGY :

Sepsis account for more than 2,00,000 deaths per year in US. Sepsis related incidence and mortality rates increases with age and preexisting co morbidity. In a survey of hospital discharge records from Seven States in 1995, Angus and Colleagues[18] estimated the annual incidence of sepsis to be 300 cases per 1,00,000 population. The estimated crude mortality rate was 28%. The median age for patients with sepsis is approximately 60 years. The attack rate in very high in infants.

A survey conducted in the Intensive Care Units in the US[3] and Europe during the year 1990 and 2000 approximately 70 to 80% of cases of severe Sepsis in adults occurred in individuals who were already hospitalized for other reasons. In 30 to 50% cases no definite etiology was found.

Sepsis caused by gram positive bacteria has steadily increased over the last two decades. *Staphylococcus aureus, CONS and Enterococci* account for approximately 30 to 50% of cases in most clinical setting. Another recent trend is the emergence of fungi particularly candida as

etiological agents in blood stream infections. *Candida spp*. caused 5 to 20% of sepsis cases[2].

PATHOPHYSIOLOGY OF SEPSIS:

Infection is initiated when bacteria penetrate host barriers like skin and mucosa. Depending on the virulence of infecting agents, immune status of the patients, local host defense mechanism is overwhelmed, leading to microbial invasion of the bloodstream. Sepsis is characterized by loss of hemostatic balance and endothelial dysfunction, which in turn severely compromise the cardio circulatory system as well as intracellular hemostasis. Cellular hypoxia and apoptosis then contribute to organ dysfunction and death.

PATHOPHYSIOLOGY OF SEPSIS IN SCHEMATIC ORDER[7,20] :

- Microbial stimulus
- Host immune response in sepsis
- Loss of hemostatic balance
- Endothelial dysfunction
- Cardiac and circulatory dysfunction (microcirculatory dysfunction)
- Endocrine dysfunction
- Tissue hypoxia
- Apoptosis

MICROBIOLOGICAL STIMULUS

Gram Negative Sepsis:

In gram negative bacteremia, initiation of the immune response is mediated by LPS (Lipopolysaccharide), a bacterial cell wall product. In plasma, LPS is bound to LPS binding protein (LBP). Bound LPS is transported to opsonic receptor CD14, which is located on several cell membranes including on monocytes[18]. A soluble form of CD14 interacts with CD14 negative cells. (eg Dendritic cells). However, CD14 alone cannot explain the action of LPS, because CD14 does not have an intercellular tail.

Another binding site of LPS in transmembrane receptor TLR 4 (Toll Like Receptor), which exist in combination with the accessory protein MD2[15].The binding of LPS to CD14 and TLR 4, induces via other molecules activation of the transcription factor, nuclear factor kappa B (NF-kB). Activated kB migrates into the nucleus where it binds to and activates gene promoters, resulting in the transcription and expression of genes for cytokines and other proinflammatory mediators[17]. In monocytes LPS also induces cytokine transcription via the triggering receptors expressed on myeloid cells-1 and the myeloid DAP-12 associated lectin[14]. Intracellular pattern recognition protein in monocytes for LPS has recently

been identified as another pathway of cytokine expression and include nucleotide binding oligomerization domain 1 & 2 as LPS binding sites[20].

Gram Positive Sepsis

During the last decade, gram positive bacteria have gained greater importance as causative organisms for sepsis[19]. They lack endotoxin and are recognized by cell wall components such as peptidoglycans and released bacterial toxins (exotoxins). Recently, LTA (Lipotechoic acid), a component of the cell wall in all gram positive bacteria, has been recognized as main pattern for recognition of gram positive bacteria. TLR2 has been identified as the only pattern recognition protein for gram positive bacteria[18]. TLR 2 is not a specific receptor for LTA. Clinically gram positive sepsis and gram negative sepsis are not distinguished. Peptidoglycans and LTA stimulate the release of TNF α, IL 6 & IL 10.

BLOODSTREAM INFECTIONS:

Microorganisms enter the blood stream by various mechanisms and lead to complications like shock, multiple organ failure, disseminated intravascular coagulation (DIC) and death. The microbial agents causing bacteremia are bacteria, fungi, viruses and parasites[2].

TYPES OF BLOOD STREAM INFECTIONS :

Bacteremia can be transient, intermittent or continuous

1. **TRANSIENT BACTEREMIA** : This occurs when organisms (alters members of normal flora) are introduced into the blood stream, by minimal trauma to membranes (eg brushing of teeth, straining during bowel movements, medical procedures[21]).
2. **INTERMITTENT BACTEREMIA**: This occurs when bacteria from any infected site are periodically released into the blood. (eg. Abscess, colitis, infection of body cavities)
3. **CONTINUOUS BACTEREMIA**: This occurs when infection is intravascular like infected endothelial surface (endocarditis or aneurysms), infected devices (AV fistulas, indwelling cannulas, intra arterial catheters[19])

Bacteremia can be primary or secondary

1. **PRIMARY BSI:** BSI is called primary if the point of entry of infection or focus cannot be determined or if it arises from an intravascular catheter [catheter related BSI (CRBSI)].
2. **SECONDARY BSI:** BSI is called secondary if any distant site other than an Intravenous catheter is established as the portal of entry or origin.

Bacteremia can be community acquired or nosocomial depending on epidemiological settings[20].

1. **COMMUNITY ACQUIRED BACTEREMIA**: It is detected within 48 hours of admission and the patients should not be hospitalized within previous 30 days and there should not be any recent history of invasive procedures (eg Foley catheter, IV catheter, Central venous catheter or dialysis)
2. **NOSOCOMIAL ACQUIRED BACTEREMIA**: It is detected after 48 hours of admission in hospital and is associated with long term hospital stay, invasive procedures, long term antibiotic therapy.

The major classification of blood stream infection are

1. **INTRAVASCULAR INFECTION**: Infection that originates within cardiovascular system contributes to intravascular infection. It includes infective endocarditis, Mycotic aneurysm, Catheter related bacteremia and Suppurative thrombophlebitis. These infections are life threatening and leads to serious illness.
2. **EXTRAVASCULARINFECTION** : Here the bacteria invades the circulation through the lymphatic system. Most cases of clinically significant BSI are due to extravascular infection.

The most common routes of extravascular infection are genitourinary tract (25%), respiratory tract (20%), abscesses (10%), surgical wound infection (5%), biliary tract (5%) and other unknown sites(25%) .In one third of bacteremia source of infection is not identified. Organisms causing BSI through extravascular sites are members of the family Enterobacteriaceae, Streptococcus pneumonia, Staphylococcusaureus, anaerobic cocci, Neisseria gonorrhoeae, Clostridium spp, Bacteroides, Beta hemolytic Streptococci and Pseudomonas.[2]

RISK FACTORS:

The risk factors & underlying conditions of BSI are immunosuppression, irrational use of antibiotics that leads to emergence of resistance to drugs, invasive procedures that allow microorganisms to enter the host, surgical procedures, underlying organ failure and Malignancy[15].

CLINICAL FEATURES:

The clinical features or presentation ranges from mild symptoms occurring from transient bacteremia to fulminant sepsis leading to Septic shock, DIC, high mortality and lifethreatening complications.Continuous bacteremia is associated with endocarditis (intravascular) or other extravascular infection like typhoid fever (for first week) or brucellosis. Transient bacteremia occurs following any minor surgeries or manipulation.

Intermittent bacteremia is commonly secondary to any local abscess. Fever is the most common presenting symptoms in almost all patients with intermittent and continuous bacteremia. Other clinical features include increased respiratory rate, heart rate and decreased blood pressure. Bryan emphasized that patients with positive blood cultures are 12 times more likely to die during hospitalization than patients with negative blood cultures[23].

MICROBIAL PATHOGENS IN BSI:

In recent studies done by Pittet et al, Valles et al 2009, about 50-60% were caused by gram negative organisms and 20-30% by gram positive organisms. Fungi mainly candida contribute to 6-10% of episodes. The most common organisms in BSI are *Coagulase negative staphylococci, Staphylococcus aureus, Enterococci, Candida spp, E.coli, Klebsiellaspp, P.aeruginosa, Enterobacterspp, Acinetobacter spp*[27].

STAPHYLOCOCCUS AUREUS:

Staphylococcus aureus is a part of normal humanflora. The anterior nares is the common site of human colonization but it can also be seen in the skin (when damaged), vagina, axilla, perineum and Oropharynx. 25-50% of healthy persons are usually colonized with Staphylococcus aureus. The colonization rate is higher in immunosuppressed conditions. It is the leading

cause of nosocomial infection. *Staphylococcus aureus* is the most common cause of surgical wound infections. It is the second most common cause of primary bacteremia next to CONS[15]. Nosocomial organism is multi drug resistant. Methicillin resistance now commonly called as MRSA was first reported by Barber in 1961. From then MRSA was a major clinical and epidemiological problem in hospitals and critical care settings. MRSA BSI is most commonly seen in ICU setup and Hospital acquired infections. Although among the community acquired infections *S.aureus* is an important cause of skin and soft tissue infection, respiratory infections and IV drug users[20]. Small colony variants (SCV), subpopulation of *S.aureus* that responded poorly to chemotherapy were identified (Seifert et al 2003).

COAGULASE NEGATIVE STAPHYLOCOCCI (CONS)

BSI due to CONS is mostly nosocomial but 8-10% are community acquired (Diekema et al 2003) . Over the past several years CONS was considered to be contaminants with little clinical significance. For the past four decades they have become important agents of human disease mainly in nosocomial condition dueto invasive procedures. CONS should be reported in BSI if two or more blood culture positivity is obtained[10]. *S.epidermidis* is the most common isolate among CONS other organisms occurring in BSI are *S.hemolyticus, S.capitis, S.schleferi, S.warneri, S.saprophyticus and S.lugdunensis*[19].

ENTEROCOCCOUS SPP:

These organisms are normal flora of GI tract and biliary tract, less commonly of vagina and male urethra. They are now considered important agent of human disease, because of their resistance to antimicrobial agents to which other streptococci are generally susceptible. They are second most common cause of nosocomial UTI and wound infection and third most common cause of nosocomial bacteremias[19]. Due to the emergence of Vancomycin resistant enterococci[26] (VRE), they are associated with serious super infections among patients receiving broad spectrum antimicrobial chemotherapy. GI enterocccal bacteremia usually results from GI tract and genitourinary tract infections. They are common cause of prosthetic valve endocarditis and constitute 5-20% of cases of endocarditis[25] . Most common species is *enterococcus faecalis*[21].

OTHER GRAM POSITIVE ORGANISMS:

S.viridans, S pneumonia, Listeria monocytogens andDiptheroids.

GRAM NEGATIVE ORGANISMS:

E.COLI, KLEBSIELLA AND OTHER ENTERO BACTERIACEAE:

E.coli and *Klebsiella spp.,* are the most common cause of both community acquired and nosocomial acquired bacteremias.

Enterobacteriaceae accounts for 17% of all BSI and they are the second common cause of BSI next to CONS [19]. 20% of hospitalized infections are due to Klebsiella pneumoniae which causes pneumonia. In healthy and immunocom promised patients non typhoid salmonella spp is important cause of BSI. Emegence of ESBL mostly due to E.coli and Klebsiella spp are clinically significant in management of sepsis.

PSEUDOMONAS AEURGINOSA:

BSI due to Pseudomonas aeurginosa is mainly nosocomial contributing significant morbidity and mortality of hospitalized patients. The prognosis is poor leading to life threatening complications and septic shock[27].

OTHER GRAM NEGATIVE PATHOGENS:

Acinetobacter spp., Haemophilus influenza, Neisseria species

Fungi:

Blood cultures remain an important diagnostic tool for disseminated fungal infections. Lysis centrifugation system is used now days to detect filamentous fungi causing sepsis. Among fungi, *candida albicans* is most frequently isolated from the blood leading to 10% of all nosocomial infections[20]. Candida infection is usually associated with malignancy, neutropenia, HIV/AIDS and other immunosuppressive conditions.

LABORATORY DIAGNOSIS OF BLOOD STREAM INFECTIONS

Blood cultures are important diagnostic tool in patients with conditions that predispose to BSI[2]. The growth of bacteria can be detected using manual techniques and automated methods. Many automated systems are available now which gives rapid results. Once growth is isolated, the organism is identified and tested for its susceptibility to various antimicrobial agents[20].

SPECIMEN COLLECTION & TRANSPORT:

Blood cultures are obtained using a sterile needle or syringe. About 5-10 ml of blood should be drawn aseptically by single veni puncture, inoculated into the blood culture bottle containing medium and incubated. After18-24hrs of incubation the bottles are checked for presence of microorganisms.Blood cultures should not be obtained from indwelling intravascular catheters as there is greater risk of recovering skin organisms. If indwelling catheters are considered as source of BSI, then blood samples are collected from the catheter site.[2]

SITE OF COLLECTION:

Since there is increased incidence of bacteremia from bacteria that are part of normal skin flora such as *Coagulase negative staphylococci, Corynebacteria and Bacillus species,* appropriate asepsis should be

followed while collecting samples of blood. Blood is collected from peripheral vein (eg. cubital vein) .Contamination is more in femoral vein and arterial blood are of no use in recovering pathogens (Tenney et al, Vaisanen et al. 1985). The rate of contamination is higher from IV catheter blood samples since colonizers present in catheter gives false positive results. Infact catheter blood sample is useful when catheter related blood stream infection (CRBSI) is considered[2].

ASEPTIC PRECAUTION:

The skin site over the vein is disinfected with 70% isopropyl alcohol in a circle rubbed vigorously. Then from the centre of the circle, 2% tincture of iodine (or povidone-iodine) is applied in circles and allowed to dry on the skin for at least 1 minute. Gloves should be used by the person collecting the blood. It is important to use both alcohol and iodine compound to disinfect the venipuncture site[5,11].

TIME OF COLLECTION

Blood should be collected during febrile episodes or as soon as after the onset of fever and chills. It is also essential to collect blood samples before starting antibiotic therapy or end of a dosing interval[29].

SPECIMEN VOLUME

Adults: In adults with BSI the colony forming units (CFU) per milliliter of blood is very low. Therefore a sufficient sample volume of blood is required for the successful detection of bacteremia. The rate of isolation is greater, when more blood is cultured[29]. Results from a study suggested that the yield increases by 3.2% for every milliliter of blood cultured. For adults 10-20mL of blood per culture is required to increased the yield by 30 percent[5].

Children: It is unsafe to obtain large volumes of blood from children, particularly infants. Inspite of low level of bacteremia in infants and children it is safe to obtain as much as 4% to 4.5% of patients blood volume for culture. So the relationship between blood volumes for culture from infants and children by Baron and colleagues[30] is shown in (Table 1).

Table 1 : Blood Volumes Suggested for Cultures from Infants and Children

Weight of Patients	Recommended volume of blood for culture (mL)				
Kg	**Total blood volume**	**Culture No 1**	**Culture No 2**	**Total volume for culture**	**% of total blood volume**
≤ 1	50-99	2		2	4
1.1-2	100-200	2	2	4	4
2.1-12.7	> 200	4	2	6	3
12.8-36.3	> 800	10	10	20	2.5
> 36.3	> 2200	20-30	20-30	40-60	1.8-2.7

NUMBER OF BLOOD CULTURES

The rate of detection increases with the number of blood cultures .The first blood culture should be obtained at the same time and inoculated into two different media and at two different temperatures . The second set of culture should be obtained in the same way & this increases the sensitivity rate to 99%[4,5,8,30]. There is no current recommendation for ideal time difference between two blood culture[10].

CULTURE MEDIA:

The media used for Blood cultureshould be nutritionally enriched with Tryptic or Trypticase soy, brain heart infusion, Columbia Agar and Brucella broths are used commonly. These commercially available media contains the anticoagulant Sodium ployanethol sulfonate (SPS, Liquoid) (Wilson et al. 1994), 0.025% to 0.05% concentration[19]. Bacteria cannot survive well in the clot and so anticoagulants are used. SPS inactivates neutrophils &inhibit antibiotics including Streptomycin, Kanamycin, Gentamycin, and Polymyxin. The side effects of SPS is that it inhibits the growth of certain bacteria like *Neisseria gonorrhoea, Neisseria meningitidis, Peptostreptococcus anaerobius*[2]. This inhibitory effect of SPS can be neutralized by addition of gelatin (1%) to the medium. The use of resin in blood culture media significantly increases the recovery of pathogens such as members of *the family Enterobacteriaceae, Enterococcus spp, S.pneumoniae and S. viridans.* Antimicrobial agents normally present in human blood are neutralized by adding adsorbents such as resins, activated charcoal and fuller'searth(Peterson et al 1983)[19].

TRANSPORT

Refrigeration of blood cultures are not recommended. Rapid transportation of blood cultures and immediate processing in the laboratory is done for appropriate recovery of pathogens[31].

SAMPLE PROCESSING

Processing of blood cultures includes incubation, gram staining and subcultures.There are manual and automated blood culture systems available for processing.

SYSTEMS FOR PROCESSING BLOOD CULTURES:

MANUAL BLOOD CULTURE SYSTEMS:

The two commercially available manual Blood culture systems are variations of classic biphasic media bottles known as Castenada bottles. They are

The Oxoid Signal System :

The Oxoid (Ogelensburg, N4) signal system is a single bottle blood culture system where the bacterial growth is determined by the production of CO_2.The blood culture bottle is connected to a second plastic chamber, called signal chamber fitted at the bottom with a long needle.

Bacterial growth and metabolism produces CO_2. Weinstein et al designed new bottle with increased head – gas space which increased the yield of organisms[32].

BBL Septi – Check Blood Culture System :

The Septi-Chek biphasic agar slide system (BD Diagnostic system, sparks, MD) uses blood culture bottle containing brain heart infusion broth or trypticase Soy broth[33]. The slide contains paddle with agar surface. After inoculation, the plastic contained "slide" is screwed on .The agar surface is flooded with the broth for few minutes and then again placed upright for continuous incubation. The bottle is inverted at regular intervals and sub cultured after incubating at 37 c for 4 - 6hrs .

LYSIS CENTRIFUGATION BLOOD CULTURE SYSTEM :

(Wampole Isostat / Isolator Microbial System)

The isolator microbial system (Wampole laboratories, Princeton, NJ) is a special tube contains Saponin,a chemical which lyse the white and red blood cells, Propylene glycol to decrease foaming, SPS as an anticoagulant, EDTA to chelate calcium and a small amount of Fluorochrome[19]. This is an alternative blood culture method used for recovery of fastidious (or) slow growing organisms *(Bortonella henselae)*[19], *Filamentous molds, dimorphic fungi, Malassezia furfur* and

Legionella spp.,[2] The mean recovery time of yeasts and *Histoplasma capsulatum* is reduced from 4.9 days and 24.14 days to 2.12 and 8 days respectively with the isolator[19]. Increased rate of contamination is the major problem with isolator system and it can be decreased by using dry agar plates, proper disinfection of work area and sample processing in a Laminar hood.

EXAMINATION OF MANUAL SYSTEMS:

Blood culture bottles are incubated at 37 C for 16to 18 hrs and examined for hemolysis, production of gas, or turbidity. Blind subcultures are made after 24 hrs of incubation and microscopic examination should be performed. For microscopic examination Gram stain or Acridine orange stains are used. Acridine orange stains detect 10^4 CFU/ml whereas Gram stain detects 10^5 CFU/ml[34]. Tierney et al reported 16.8 percent increase in the detection of bacteremia using acridine orange stain while the broth is macroscopically negative.

AUTOMATED AND COMPUTERIZED BLOOD CULTURE SYSTEMS

The first automated system was BACTEC 460 (Becton dickinson), was introduced in the 1970s. The results are rapid and obtained within a day

After positive culture is obtained, bottles are removed for gram stain and sub culture[2].

BACT/ALERT MICROBIAL DETECTION SYSTEM:

This system contains CO_2 sensitive chemical sensor separated by unidirectional CO_2 permeable membrane which is bonded to the bottom of every bottle. The growth of microorganisms in the blood broth produces CO_2, which makes the color sensor to turn from green to yellow.

THE BACTEC9240/9120 BLOOD CULTURE SYSTEM:

This system is similar toBACT/Alert but the only difference is the use of fluorescent, rather than spectral light for detecting change in CO_2 concentration.

THE TREK ESP CULTURE SYSTEM

The ESP blood culture system (TREK diagnostic system, Cleveland, OH) is different from the above two systems by

1. The CO_2 production, monitored manometrically.
2. Monitoring both gas consumption and production.
3. In addition to CO_2 production H_2 and O_2 concentration changes are also monitored (testing multiple gas production[27]).

ANTIMICROBIAL SUSCEPTIBILITY TESTING:

After identification of the causative organisms the management of BSI includes early and appropriate treatment by antimicrobial therapy.

Antibacterial susceptibility testing:

Antibiotic sensitivity testing was done by Kirby Bauer disc diffusion method, using 0.5 McFarland's turbidity on Mueller Hinton agar plates Commercially available Hi-media antibiotic discs are used.

Antifungal susceptibility testing:

It was performed on Mueller Hinton agar plate supplemented with 2% glucose and 0.5 µg/ml methylene blue. Commercially available Hi-media antifungal discs are used.

MANAGEMENT OF BSI

According to Bayer et al, the general treatment principles to be followed:

1. High dose parenteral antimicrobial therapy is recommended to reach sustained antibacterial activity;
2. Prolonged administration of antimicrobial therapy is required to prevent relapse;

3. Bactericidal agents are generally preferred over bacteriostatic agents;

4. Combination therapy is recommended to produce a rapid bactericidal effect. The choice of antibiotics for the treatment is governed by susceptibility of the causative organism. Determination of minimal inhibitory concentration is recommended to define optimal treatment.

MATERIALS AND METHODS

STUDY PERIOD:

This is a cross sectional study undertaken over a period of 1 year from October 2010 to Sep 2011.

STUDY PLACE:

The study was carried out at the Institute of Microbiology, Madras Medical College, RGGGH, Chennai-3.

ETHICAL CONSIDERATIONS:

This study was reviewed and approved by Institutional ethical committee Madras Medical College and RGGGH, Chennai-3. Informed written consent was obtained from the study population or their guardians after providing full explanation of the study. All patients satisfying the inclusion criteria were documented and interviewed by structured questionnaire.

STATISTICAL ANALYSIS:

Statisticalanalysis were carried out using statistical package for social science (SPSS) and Epi-Info software by a statistician. The proportionaldate of thiscross sectional study were tested using pearson'schi square analysis test and Binomial proportion test.

STUDY GROUP:

Blood samples from inpatients admitted in IMCU of RGGGH, Chennai.

SAMPLE SIZE:

All consecutive septicaemic patients admitted in IMCU during the study period.

INCLUSION CRITERIA:

1. Patients above 15 yrs[2]of age group.
2. Patients with known Sepsis or strong clinical suspicion of sepsis
3. Signs of SIRS[16].
4. Dysfunction of atleast one organ system[15].

SIRS CRITERIA:

SIRS criteria and proven (or) suspected infection is called sepsis. The criteria includes[3]

i. Temperature > 38˚C or < 36˚C

ii. HR >90/min

iii. RR >20/min

iv. WBC count $\geq$ 12,000/cu.mm or < 4000cells /cu.mm or 10% immature (band) neutrophil forms.

All patients satisfying the inclusion criteria were only documented and were assigned serial numbers. Patients were interviewed by structured questionnaire and their hospital records were used to know about their past medical conditions. They were followed up prospectively until discharged or after admission.

EXCLUSION CRITERIA:

1. No clinical suspicion of sepsis
2. Prior antibiotic administration

CASE DEFINITION:

SEPSIS:

SIRS with presumed or confirmed infectious process. This is the host response to a microbiological event.

BACTEREMIA:

BSI was diagnosed when a blood culture grew an organism with (2˚ BSI) or without (1˚ BSI) any obvious focus of sepsis – True bacteremia was considered when one or more blood cultures showed a recognized pathogen.

COMMUNITY ACQUIRED BACTEREMIA is defined if the first positive blood culture was obtained before or within 48 hrs of hospitalization and not associated with any procedure performed after hospital (or) ICU admission.

NOSOCOMIAL BLOOD STREAM INFECTION is defined in a patient with significant blood culture positive for a bacteremia (or) fungus that is obtained more than 48 hrs after admission to the hospital and is directly related to invasive procedures on admission to the hospital (eg Urinary catheterization or insertion of IV line).

The source of BSI was identified by the isolation of same pathogenic organism from both the source and the blood.

The following definitions were used to categorize the **source** of bacteremia.

1. **Pneumonia** is defined as the presence of an acute illness associated with respiratory symptoms and an infiltrate on chest roentgenography.
2. **Cellulitis** is considered if physical finding of an erythema, tenderness, and warmth within a focal distribution is found.
3. **Urinary tract infection** is considered if urine culture was positive and no other source of blood stream infection was found.

4. **Endocarditis** is defined as the demonstration of valvular vegetations on echocardiography, evidence of septic emboli or a new murmur and absence of any other source of septicaemia.
5. **Phlebitis** is defined as an inflammation around a venous line from catheter in place for at least 72 hours & positive blood culture in the absence of another source of bacteremia.
6. **Catheter related blood stream infection (CRBSI)** is diagnosed when blood culture obtained from a peripheral vein and CVC grow the same organism and the CVC tip with quantitative bacterial counts >15 CFU (Maki method).

Data collection:

History was obtained from all patients and physical examination was also done. Information on antimicrobial therapy before admission was recorded.

Sample collection and processing:

Collection:

Blood cultures were obtained using aseptic technique. Venipuncture sites were disinfected before phlebotomy with 0.5% Chlorhexidine solution followed by 70% isopropyl alcohol.[11]

Persons collecting the specimens should replace the needle used for the venipuncture with a fresh sterile needle before inoculating the blood into the culture bottles. It should be noted that they should hold needle by its butt, not the shaft and with sterile forceps or with gloved fingers.[4]

Processing:

1st set : For the purpose of culture, 10-20ml of blood was collected (to get dilution of 1:5 or 1:10) and one half of the sample was inoculated into biphasic BHI (Brain heart infusion) media and other containing trypticase soy medium[28].

2nd Set: Within 12 hours second set of samples were collected in the same way.

Blood culture bottles were incubated at 37˚C for 24 hrs. Subcultures were done at the appearance of turbidity, gas production or the presence of microcolonies over the clot at 24 hrs, blind subcultures irrespective of turbidity or gas production is done at 48 hrs and final subculture were done at the end of 7th day, as follows:

a. On 5% sheep Blood agar aerobically
b. On 5% sheep blood agar anaerobically
c. On MacConkey agar aerobically
d. Nutrient agar
e. On Chocolate agar in CO_2, all incubated at 37˚C for 24 hrs.
f. Two Sabouraud's dextrose agar tubes incubated at 25˚C & 37˚C.

IDENTIFICATION OF CULTURE:

Morphology of colonies grown on blood agar, MacConkey agar, Chocolate agar at 18-24hrs were noted and isolates were identified by

1. Gram stain – to identify whether gram positive or gram negative organisms.
2. Gram positive cocci were proceeded with calatase and coagulase tests.
3. Hanging drop done to find out motile and non-motile organisms.
4. Preliminary tests like oxidase, catalase tests were also performed.
5. Members of the species were identified based on biochemical parameters using IMVIC reactions and other sugar fermentation tests.

Antimicrobial susceptibility tests:

Antimicrobial susceptibility testing of the bacterial isolates were performed using Kirby –Bauer disc diffusion method on Mueller – Hinton agar and zone diameters were interpreted according to the CLSI guidelines.[36]

Medium used	:	MHA (Mueller – Hinton blood agar) plate
Inoculum	:	0.5 McFarland turbidity, lawn culture
Incubation	:	37˚C ambient air, incubated for 16-18 hrs

Preparation of Inoculum:

About 4-5 colonies of similar morphology were picked up with the help of straight wire and inoculated in 5ml of peptone water, and it is incubated for 3-5 hrs which gives 0.5 McFarland's turbidity.[2] A sterile cotton swab was taken and dipped into it and pressed firmly against the wall of the test tube so as to remove the excess broth from the swab.

Mueller Hinton agar plate was dried and swabbed in three directions approximately 60˚ each time to ensure complete distribution of the inoculums over the entire plate. The antimicrobial discs were dispensed on the Mueller Hinton agar plate using dispenser and pressed down to give complete contact with the surface of the agar. The discs were distributed evenly on the plate and the distance should not be closer than 24 mm from centre to centre. Not more than 6 discs were placed in a single plate[37]. After an incubation period of 16-18 hrs each plate was examined. The diameter of the zones which gave complete inhibition was measured. The diameters of zones of inhibition was interpreted according to CLSI standards for each organism.

The following standard strains were used

1. Staphylococcus aureus – ATCC 25923
2. Escherichia coli – ATCC 25922
3. Pseudomonas aeruginosa – ATCC 27823

The panel of antibiotics included in the antimicrobial sensitivity testing for Gram negative bacilli were (Himedia, Mumbai).

INTERPRETATIVEZONE DIAMETERS FOR GNB

Antimicrobial	**Disc content**	**Inhibition zone in mm**		
		Resistance	**Intermediate**	**Sensitive**
Amikacin	30 µg	14	15-16	17
Cefepime	30 µg	14	15-17	18
Ceftazidime	30 µg	14	15-17	18
Cefotaxime	30 µg	14	15-22	23
Ciprofloxacin	5 µg	15	16-20	21
Gentamicin	10 µg	12	13-14	15
Imipenem	10 µg	13	14-15	16
Ofloxacin	5 µg	12	13-15	16
Piperacillin/ Tazobactam	100/10 µg	17	18-20	21

The panel of antibiotics included in the antimicrobial sensitivity testing for Gram positive cocci are given below (Himedia ,Mumbai).

INTERPRETATIVE ZONE DIAMETERS FOR GPC

Antimicrobial	**Disc content**	**Inhibition zone in mm**		
		Resistance	**Intermediate**	**Sensitive**
Amikacin	30 µg	14	15-16	17
Cefepime	5 µg	15	16-20	21
Ceftazidime	1.25/23.75 µg	10	11-15	16
Cefotaxime	30 µg	12	13-17	18
Ciprofloxacin	2 µg	14	15-20	21
Gentamicin	10 units	28	-	29
Imipenem	5 µg	16	17-19	20
Ofloxacin	15 µg	13	14-22	23
Piperacillin/ Tazobactam	1µg	10	11-12	13

Macrobroth dilutionmethod:[38]

Minimum inhibitory concentration (MIC) for detecting Vancomycin resistance[20].

1. **Culture media**: Cation adjusted Mueller Hinton broth. (pH 7.2-7.4).
2. **Preparation of stock solution:**

Antibiotic stock solution is prepared using the formula[35]

= 1000 /p x V x C = w

Where p = antibioticpotency in relation to the base, (For Vancomycin, p=950/1000 mg; Hi-media).

V= volume of the stock solution that is to be prepared (10ml)

C= final concentration of the antibiotic stock solution (1024µg/ml)

W=weight of the antibiotic that is to be dissolved in the volume V

3. **Scheme of preparing dilution of antibiotics**

- Arrange two rows of sterile test tubes in the rack (1 row for the test & 2nd for ATCC control)

Using sterile syringe transfer 2ml of Mueller Hinton broth to the storage vial containing the working stock solution (129 µg/ml concentration). From this transfer 1ml to the first tube in each row.

- Now thereis 2ml of diluted antibiotic remaining in the storage vial . Using syringe add 2ml of MH broth to the 2ml of left over antibiotic in the, storage vial mix and transfer 1ml to second tube in each row.
- Repeat this procedure till the 8^{th} tube
- Place 1 ml of the antibiotic free broth in the last tube in each row (growth control)
- The sterility controls for the antibiotic solution is kept.

4. Inoculum preparation for the test and ATCC control and incubation:

- Take 9.9 ml of MH broth in a storage vial
- Add 0.1 ml of 0.5 McFarland turbidity matched test organism broth
- Mix well, transfer 1 ml of inoculum using 2 ml syringe to each tube containing antibiotic dilutions and also to the control tube.
- Similarly repeat the procedure for ATCC control strain
- Incubate the rack at 37ºC – overnight
- Observe the MIC of ATCC control strain, If it is out of the sensitive range, the test is invalid
- Read for the test organism
- The lowest concentration of the antibiotic in which there is no visible growth will be the MIC for the drug & for the test organism.

MINIMUM INHIBITORY CONCENTRATION TEST :

E-Test for GNB : (Epsilometer test) Triple ESBL Detection Ezy MIX™ strip, Hi-media, Mumbai.

It is a unique Phenotypic ESBL detection strip commercially available which is coated with mixture of 3 different antibiotics with & without clavulanic acid on a single strip in a concentration gradient manner. The upper half has Ceftazidime, Cefotaxime and Cefepime (Mixture +) Clavulanic acid & Tazobactum with higher concentration tapering downwards, whereas lower half is similarly coated with Ceftazidime, Cefotaxime and Cefepime (Mixture) in a concentration gradient in reverese direction.

Quality Control :

ATCC E.coli 25922

Procedure:

A Standard inoculum size of ESBL detecting strain was swabbed on MHA plate with a sterile non –toxic cotton swab, streak the entire agar surface of the plate with the swab 3 times, turning the plates at 60^0 angle between each streaking. Remove the strip with applicator provided and place the strip at a desired position on agar plate swabbed with test culture. Then the plate was incubated at $35\text{-}37^0$ for 18 hours.

Interpretation :

The Value corresponding to the point of intersection was taken as end point.

For ESBL positive Strain→ $Mix/Mix^{+} \geq 8$

For ESBL negative Strain→ $Mix/Mix^{+} \leq 8$

A. Extended spectrum β lactamases (ESBL) detection methods[35,2,19,28] :

1. Screening method :

Isolates of gram negative bacilli showing the following resistance pattern were considered to be possible ESBL producing strains[34].

Antibiotic	**Zone diameter for possible ESBL producing strain**
Ceftazidime (30 µg)	≤ 22mm
Cefotaxime (30 µg)	≤ 27mm
Ceftriaxone (30 µg)	≤25mm
Aztreonam (30 µg)	≤ 27mm

2. CLSI phenotypic confirmation method:

With the help of a sterile bacterial loop, 3-5 identical colonies were selected from a fresh overnight grown culture and inoculated into 5 ml of nutrient broth &incubated at 35ºC for 2-4 hrs and matched for turbidity with 0.5 McFarlands standard. Lawn culture of the test organism was made on to MHA plate. Antibiotic disc Ceftazidime (CAZ 30µg) and Ceftazidime/Clavulanic acid (CAZ/CA 30 µg/10mg) were placed onto the plate and incubated at 35ºC overnight. An increase in zone diameter of ≥ 5mm for Ceftazidime tested in combination with Clavulanic acid versus its zone when tested with Ceftazidime alone confirmed as ESBL producing organism[34,54].

3. Double disk diffusion synergy test:

In this test of third generation Cephalosporin discs and Augmentin (20 µg/10 µg) (Himedia, Mumbai) discs were kept 30mm apart from centre to centre on inoculated Muller-Hinton Agar (MHA). A clear extension of the edge of the inhibition zone of cephalosporin towards Augmentin disc was taken as positive for ESBL production.

B. AMPC β Lactmases detection methods:

1. Screening method:

A 0.5Mcfarland of the test isolate was swabbed on MHA plate and disc of Cefotaxime (3µg), Ceftazidime (30µg) were placed adjacent to Cefoxitin (30µg) disc at a distance of 20 mm from each other. After incubation, isolates showing blunting of Ceftazidime or Cefotaxime zone of inhibition adjacent to Cefoxitin disc or showing reduced susceptibility to either of the above drugs and Cefoxitin were considered as screening positive and selected for detection of AmpC β lactamases[39].

2. **AmpC Disc test**:

A lawn culture of E.coli ATCC 25922 were prepared on MHA plate. Sterile discs (6 mm) moistened with sterile saline (20 µl) was inoculated with several colonies of test organism. The inoculated disc was then placed adjacent to Cefoxitin disc (almost touching) on the inoculated plate. The plates were incubated overnight at 35ºC. The appearance of flattening or indentation of the Cefoxitin inhibition zone in the vicinity of the test disc is taken as positive. A negative test showed an undistorted zone.

C. Metallo β Lactamase (MBL) Detection Methods:

1. Imipenem – EDTA disc method:

A 10 µg imipenem disc (Hi Media) which contains 750 µg of EDTA solution was used. The inhibition zone with imipenem–EDTA disc were <14 mm for the MBL negative isolates and > 17 mm for MBL positive isolates.

2. Imipenem – EDTA combined disk test:

The test organism was inoculated onto MHA plate. Imipenem (10µg) and 10 µg Imipenem disc containing 750µg of EDTA solution were placed on the plate and incubated overnight. If the increase in inhibition zone with Imipenem – EDTA disc is ≥ 7mm than the Imipenem disc alone, it was considered MBL positive[40.]

D. Detection of methicillin resistance in staphylococcus aureus:

1. **Disc diffusion method:**

Colonies isolated from culture plate were suspended directly into broth, vortexed to reach 0.5 McFarlands standard. A lawn culture of the Staphylococcal colonies was made on the MHA plate and Oxacillin disc was applied. Incubation was done at 35ºC for 24 hours in ambient air. According to CLSI criteria with 1µgOxacillindisc, diameters of ≤ 10, 11-

12mm, ≥ 13mm corresponded to categorization as resistant, Suscepible dose dependant or susceptible. With 30µg Oxacillin disc diameter of ≤ 19 or ≥ 20mm corresponded to resistance or susceptibility.[2]

2. **Oxacillin screen agar:**

Oxacillin screen agar test was performed by direct colony suspension method and adjusted to match with 0.5MacFarland turbidity standard. The suspension was inoculated on Mueller-Hinton agar containing 4%NaCl and with 6µg/mlOxacillin[40]. Plates were incubatedat 35ºC for 24 hours. Any growth on the plate containing Oxacillin was considered as resistant to Methicillin.

PROCESSING OF FUNGI:

Growth in SDA is further processed by following tests:

The SDA slopes were examined after 48 hrs, 96 hrs, 5 days, 7 days, 14 days and one month for the appearance of yeasty or mouldy growth.[2]

Gramstain

Gram positive oval budding yeast cell with presence or absence of pseudohyphae.

Germtube test:

A loopful of yeast like cells obtained from SDA was suspended in 0.5mlof human serum. After incubation for 1-2 hrs, one or two drops of the suspension was placed on a clean microscopic glass slide and covered with coverslip and focussed under low and high power objective to see the characteristic germ tube formation.

CHROM agar (HIMEDIA)

A single colony was taken from sabouraud's dextrose agar and was streaked on chrom agar plate. Thenthe plates were incubated at 37˚C for 48hrs. After incubation, the plates were observed for characteristic coloured colonies of candida.

Cornmeal agar:

Another method of identification of C albicans is based on formation of chlamydospores on cornmeal agar.

A single colony from SDA was inoculated onto plate of cornmeal agar containing 1% Tween 80 and trypan blue. Three parallel streaks of about half an inch apart holding the inoculated wire about 45˚ angle and the area where cuts made were covered with sterile coverslips and incubated at 22˚C for 24hrs. After incubation the areas where cuts were made onto the

agar[41] were examined under the microscope for the presence of blastoconidia, pseudohyphae or truehyphae and chlamydospores[2]. The candida species were identified according to the morphological features on the corn meal tween 80 agar under the microscope.

Carbohydrate fermentation test:

About 0.2 ml of saline suspension of the yeast colonies were inoculated onto the different sugar media that contain durhams tube. The concentration of sugar 2% with bromothymol blue indicator and the different sugar used were glucose, lactose, sucrose, maltose respectively. The tubes were incubatedat 30 C for 48 to 72 hours[41]. After incubation period, the tubes were observed for acid and gas production.

Antifungal susceptibility test:

The antifungal susceptibility test were done by 2 methods

1. Disc diffusion method as per M 44-A document[36].
2. Microbroth dilution method, as per the CLSI guidelines on antifungal Susceptibility testing in M 27-A2 document[37].

Inoculum preparation:

About five clonies of same morphology of each atleast 1mm in diameter from 24 hr old cultures of candida spp. were picked up and

inoculated in 5ml sterile saline. The suspension was then adjusted spectrophotometrically at 530nm which matched the 0.5 McFarland's barium sulphate standard. This gives the inoculums size of 1 x 10^6 to 5 x 10^6cfu/ml. The same inoculum was used for both methods.

Quality control :

Candida albicans ATCC 90028.

Disc diffusion method:

It was performed on MHA plate supplemented with 2% glucose and 0.5 mg/ml Methylene blue[2] .Anti fungal susceptibility testing was carried out following M44-A-guidelines for clinical laboratory standards institute (CLSI) guidelines using antifungal discs.

Microbroth dilution method:

Medium[36]:

RPMI 1640 medium with glutamine, without bicarbonate in MOPS (3N- Morpholino propane sulphonic acid), buffer sterilized by membrane filtration.

Antifungal stock solution:

The MIC range of antifungal agents used were

- Amphotericin B : 0.03-16 µg/ml
- Fluconazole : 0.125 to 64 µg/ml

  Fluconazole was dissolved in sterile distilled water whereas Amphotericin B was dissolved in DMSO. From the working dilution, the desired concentrations were prepared.

Procedure:

This test was performed in a sterile, disposable 96 well microtitre plate using standard RPMI 1640 medium. After 48 hours of incubation at 35^{o}C the results are interpreted.

Interpretation:

For Fluconazole, isolates with MIC 8µg/ml were considered susceptible values, between 16-32 µg/ml dose dependent susceptible and those 64µg/ml resistant. Due to the lack of definite break points for Amphotericin B, isolates showing an MIC of $\leq$ 1.0 µg/ml were taken as susceptible and those with MIC > 1 µg/ml were considered as resistant[37].

The end point for Fluconazole was the lowest concentration in which permanent decrease in turbidity was observed. For Amphotericin B, the end

point was defined as the lowest concentration in which an optically clear well was observed.

Interpretation of Results:

A culture was considered significant when a pure isolate was isolated from a pair of blood culture bottles. The blood samples inoculated in BHI media were discarded after 7 days. Fungal blood cultures were held for 21 days before being reported as negative[20].

To be a probable pathogen:

1. Growth of same organism in repeated cultures obtained either at different times or from different anatomical sites.
2. Growth of organisms from suspected case of Endocarditis[2].
3. Isolation of commensal bacteria from patients suspected to be bacteremic. (immunocompromised pts or those having prosthetic valves[19]).
4. Growth of same organism from culture IV Catheter tip culture& the peripheral line culture confirms CRBSI.

RESULTS

A total of 150 individuals were enrolled in this study, conducted during October 2010 to September 2011 at Institute of Microbiology, Madras Medical College, Chennai. From 150 patients blood samples from all the patients were collected and subjected to culture in Brain Heart Infusion Biphasic medium and Trypticase Soy broth.

Table 2: Age Distribution of Cases (n=150)

Age	No. of cases		Total	Percentage %
	Male	Female		
16-20	9	2	11	**7.33**
21-30	18	4	22	**14.66**
31-40	23	6	29	**19.33**
41-50	35	12	47	**31.33**
51-60	15	11	26	**17.33**
>61	12	3	15	**10**
Total	**112**	**38**	**150**	**100**

Majority of the male cases belong to the age group of 41 to 50 yrs (31.33%) and the next commonest age group is 31 to 40 yrs (19.33%). Males outnumbered female in this study.

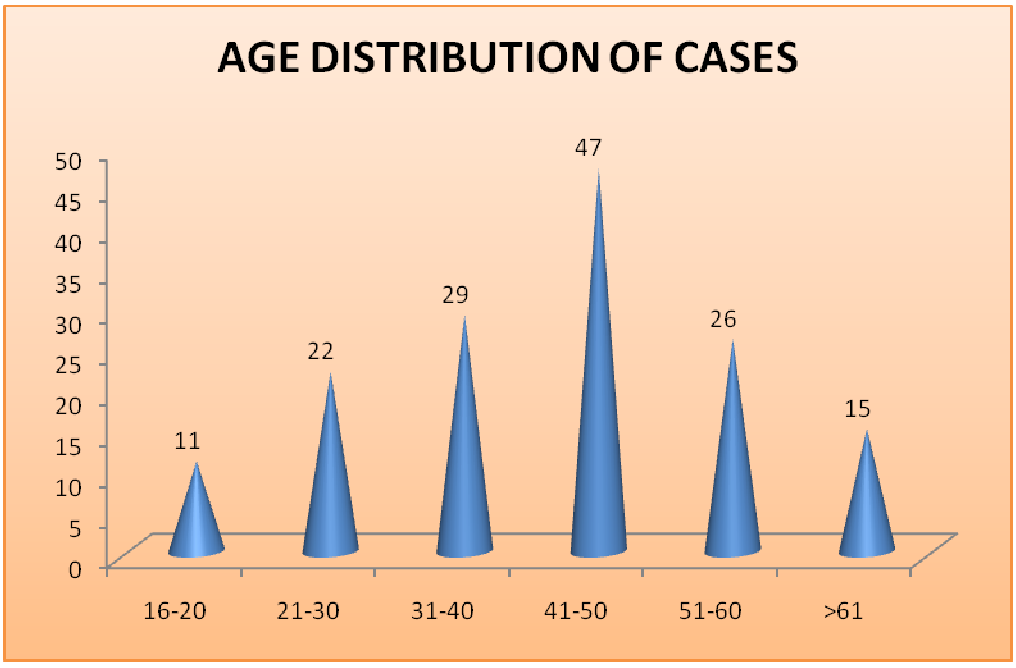


Table 3 : Sex Distribution of Cases (n=150)

Sex	No. of Cases	Percentage %
Male	112	**74.7**
Female	38	**25.3**
Total	**150**	**100**

75% of cases were males and 25% of cases were females. Males outnumbered females in this study.

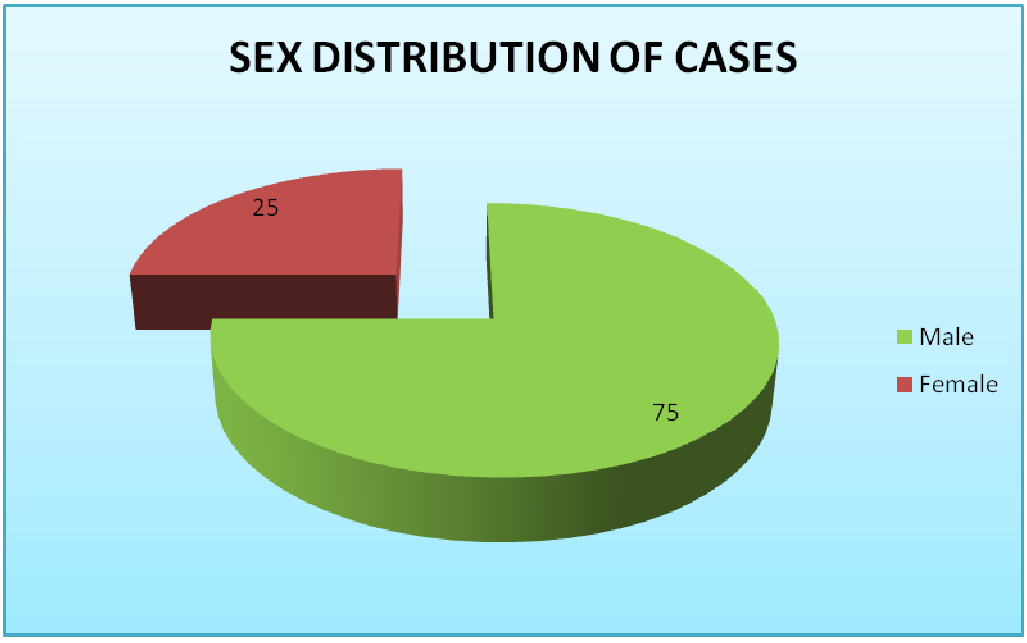


Table 4 : Correlation between Clinical Symptomatology and BSI (n-150)

S.No	Clinical Features	Cases (n=150)	%	No. of Pts with BSI	%
1.	Fever	150	100	63	100
2.	Respiratory Distress	18	12	12	19.04
3.	Ascites / Pedaledema	16	10.7	2	3.17
4.	Icterus	16	10.7	3	4.76
5.	Anemia	65	43.33	24	38
6.	Lymphadenopathy	18	12	8	12.7
7.	Neurological Symptoms*	12	8	9	14.28
8.	Urinary Symptoms	16	10.6	14	22.22
9.	Skin Lesion	15	10	9	14.28
10.	Tubercular Infection	25	16.66	10	15.87

* Includes Headache, neck stiffness, hemiparesis, hemiplegia.

Fever (100%) was present in all cases and blood culture positive cases. Anemia (43%) was the second presenting symptoms in patients with BSI. Urinary Symptoms (22%), TB infection (15.8), Respiratory Distress (19%) were next commonest symptoms in the descending order.

Correlation between Clinical Symptomatology and BSI

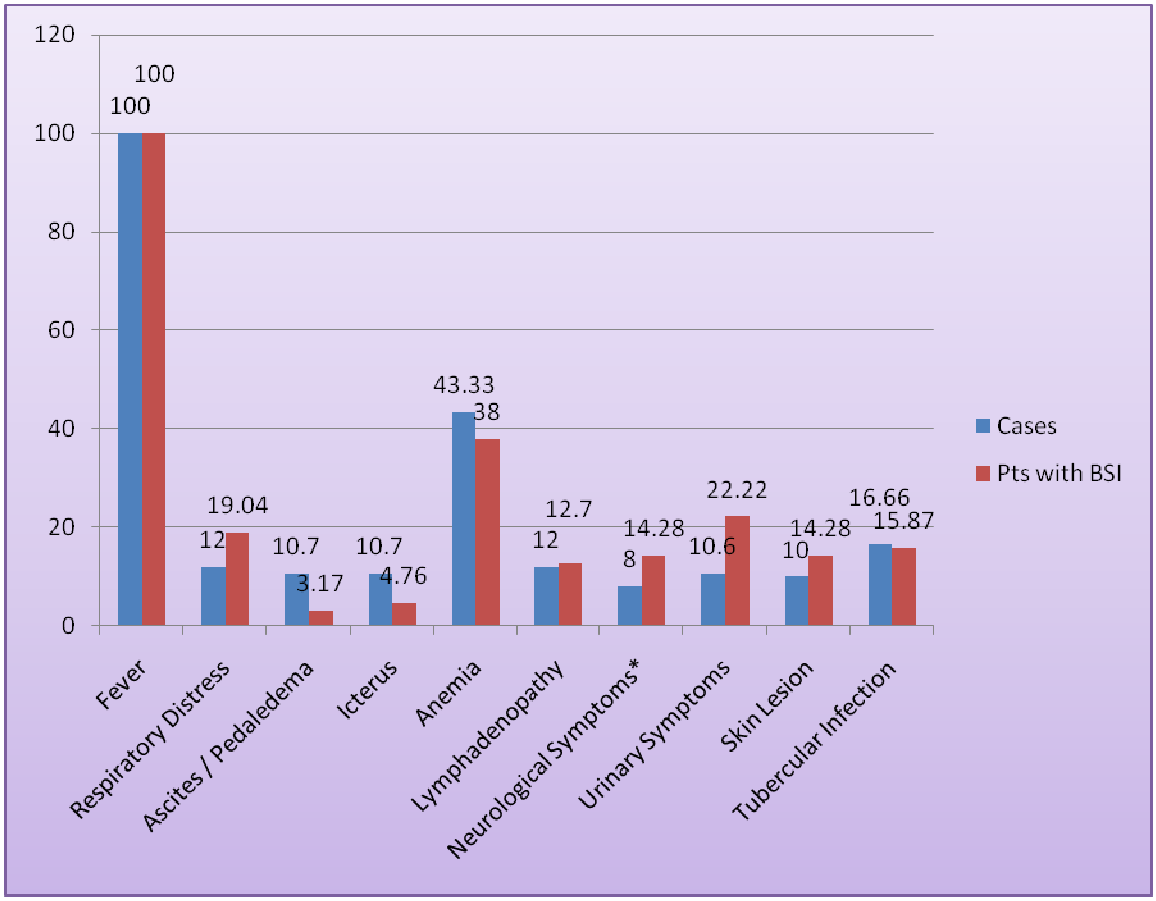


Table 5 : Results of Blood Culture (n=150)

Blood Culture	No. of cases	%
Positive	63	42
Negative	87	58

Blood culture was found to be positive in 63 (42%) cases.

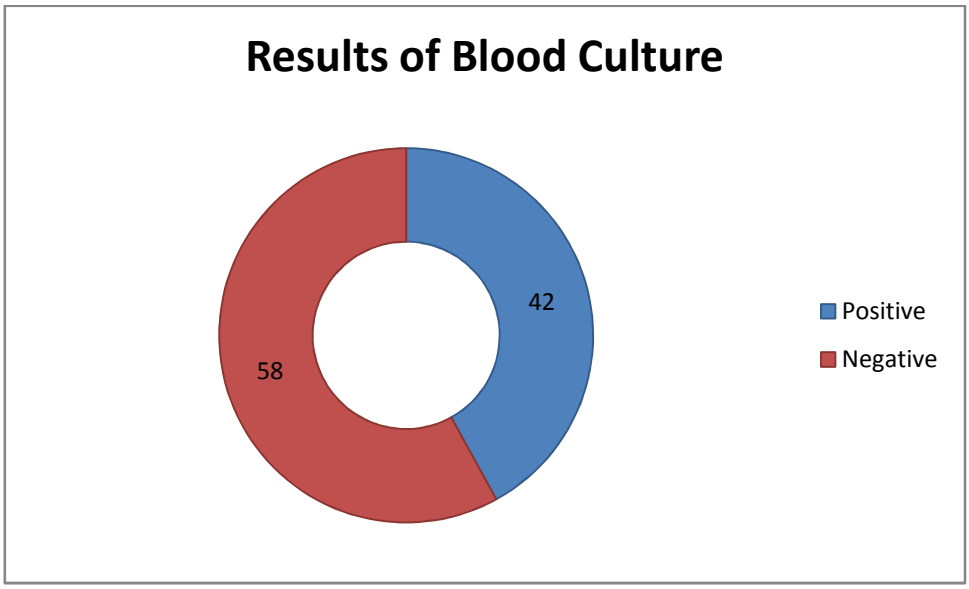


Table 6 : Age Distribution of BSI Positive Cases (n=63)

Age	No. of cases		Total	Percentage %
	Male	Female		
16-20	4	1	5	**7.93**
21-30	6	2	8	**12.69**
31-40	9	3	12	**19.04**
41-50	16	5	27	**33.33**
51-60	6	4	10	**15.87**
>61	6	1	7	**11.11**
Total	**47**	**16**	**63**	**100**

In BSI positive cases 47 were males and 16 were females. Most common age group associate with BSI was 41 to 50 years (33.33%) and the next common age group was 31 to 40 years (19.04%).

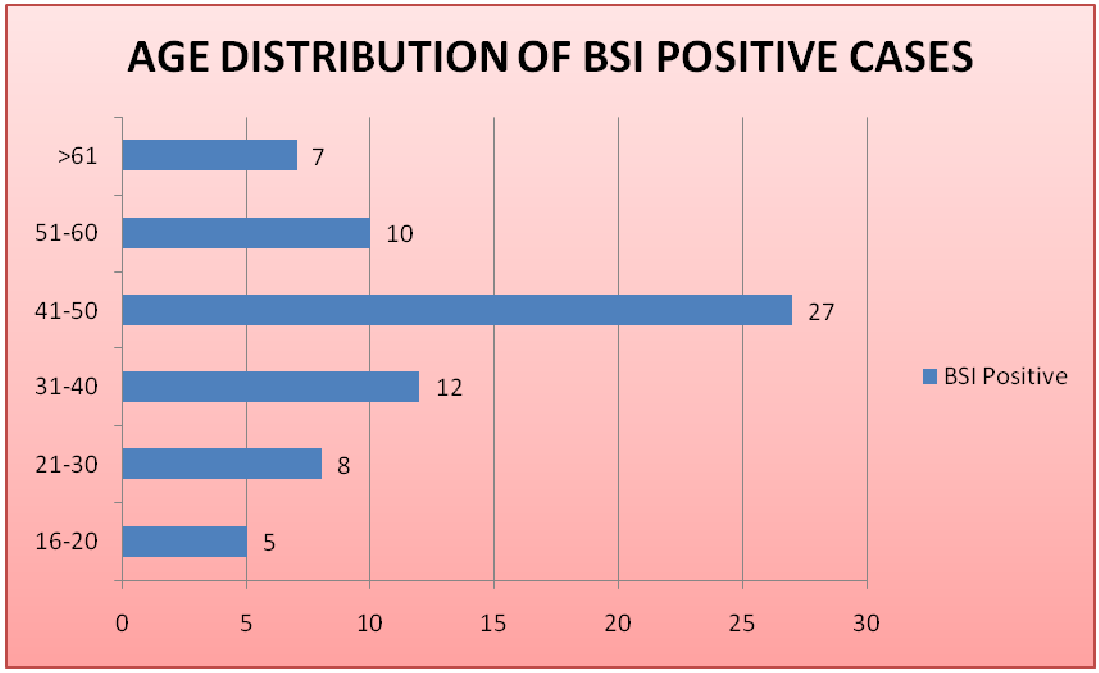


Table 7: Comorbid illness in septicaemic patients

S.No	Comorbid illness	Male	Female	Total	%
1.	Hypertension	20	8	28	18.66
2.	Diabetes Mellitus	32	18	50	33.33
3.	Asthma/COPD	18	2	20	13.33
4.	Cirrhosis	6	-	6	4
5.	Known heart disease	14	1	15	10
6.	Renal disease (acute, chronic)	18	1	19	12.67
7.	Previously treated TB	12	-	12	8
8.	Auto immune disorder	-	1	1	0.6
	Total	**112**	**38**	**150**	**100**

Most of the cases of Septicaemiais associated with Diabetes Mellitus (33.33%). Hypertension is associated with 18.66% of cases, Asthma/COPDwith 13% and Renal disease associated with 12% of cases.

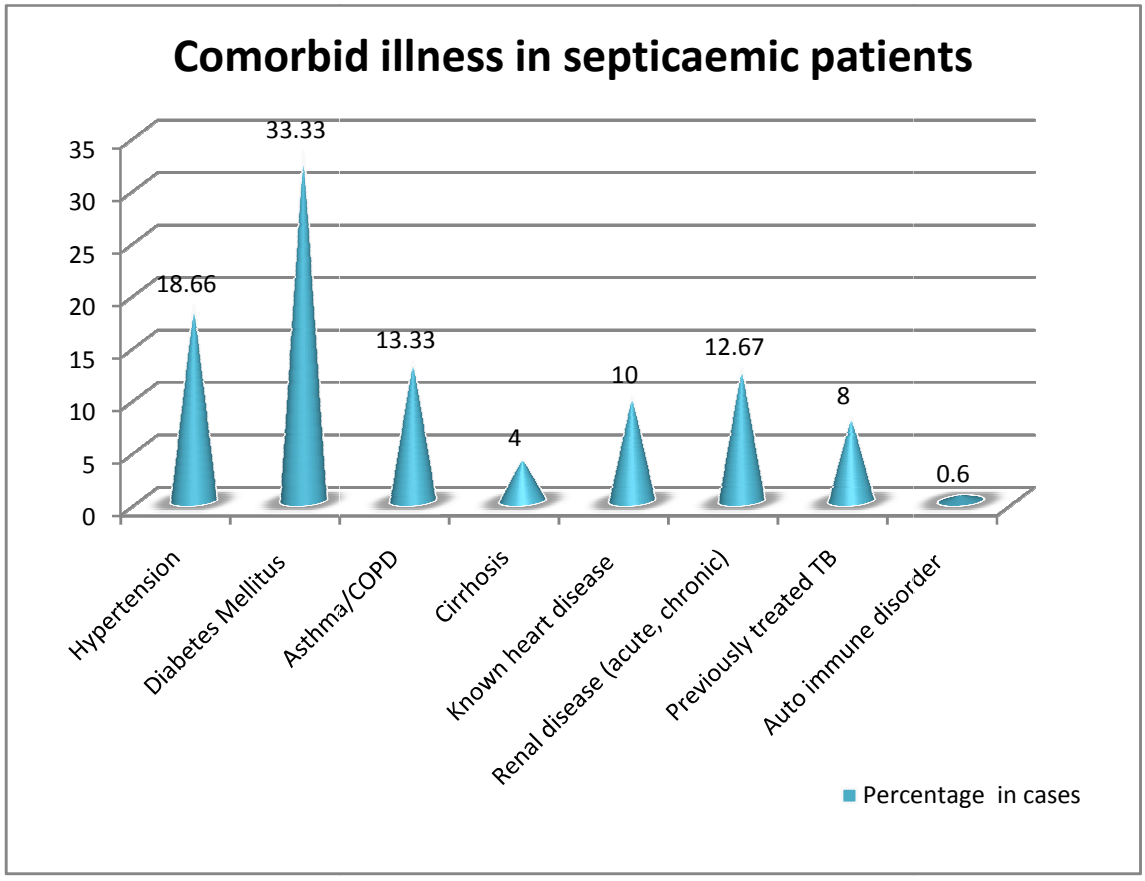


Table 8 : Epidemiology of BSI Positive Cases (n=63)

	No. of cases (n=63)	%
Community acquired	28	44.4%
Nosocomial acquired	35	55.6%

55.6% of BSI positive cases were nosocomial acquired and 44.4% were community acquired.

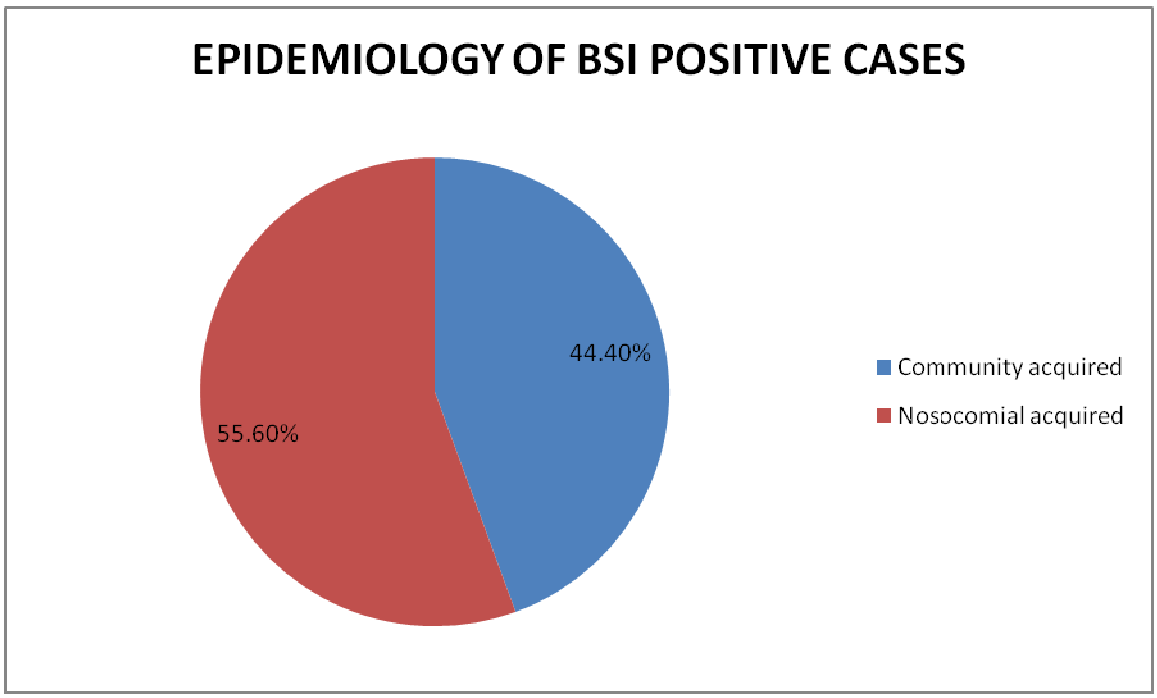


Table 9 :Organisms isolated by Blood Culture in Septicaemic Patients (n=63)

Organisms	No. of cases (n=63)	%
GRAM POSITIVE COCCI	**24**	**38.09**
Staphylococcus epidermidis	9	14.28
Staphylococcus schleiferisubsp.schleiferi	3	4.76
Staphylococcus aureus	10	15.87
Enterococcus fecalis	2	3.17
GRAM NEGATIVE BACTERIA	**35**	**55.55**
Pseudomonas aeruginosa	11	17.46
Klebsiella pneumonia	10	15.87
Escherichia coli	10	15.87
Acinetobacterspp	2	3.17
Proteus mirabilis	1	1.58
Citrobacterkoseri	1	1.58
FUNGI	**4**	**6.34**
Candida albicans	2	3.17
Candida tropicalis	2	3.17
Total	**63**	**100**

Total Culture positive cases were 63.Gram positive cocci were 24 (38.09%) , Gram negative bacilli were 35 (55.55%) & Candida spp was isolated in 4 (6.34%) of cases.

Organisms isolated by Blood Culture in Septicaemic Patients

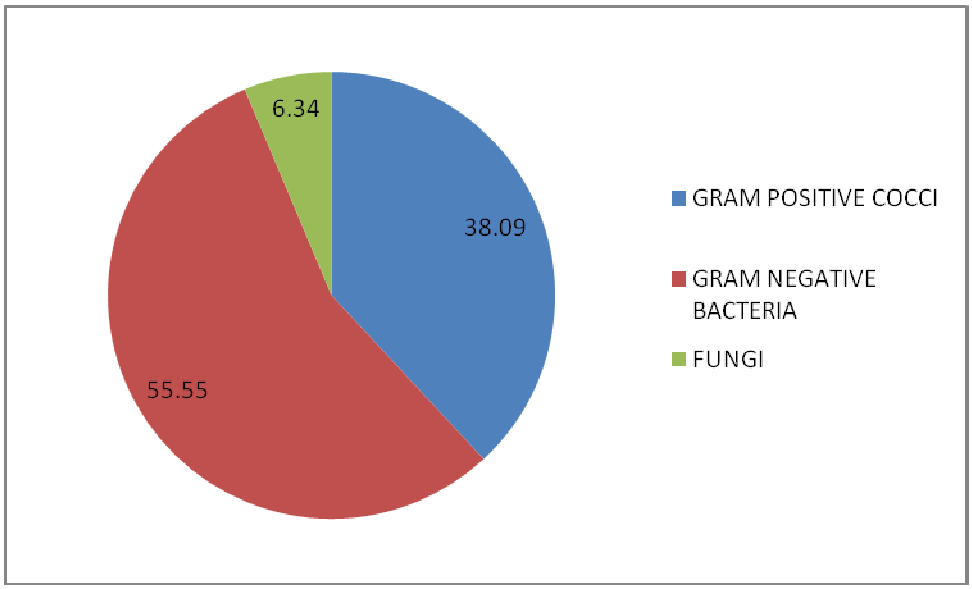


Table 10 :Antimicrobial Susceptibility of Gram positive Bacteria Causing BSI in IMCU patients

S. No	Antibiotics	Staphylococcus aureus (n=10)		Staphylococcus epidermidis (n=9)		Staphylococcus schleiferisubsp. schleiferi (n=3)		Enterococcus fecalis (n=2)	
		No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
1	AmoxycillinClavulanic acid	5	50	4	44.4	2	66.7	1	50
2	Amikacin	8	80	8	88.9	1	33.3	1	50
3.	Cephalexin	3	30	2	22.2	2	66.7	-	-
4.	Cotrimoxazole	3	30	4	44.4	2	66.7	1	50
5.	Ciprofloxacin	5	50	7	77.8	1	33.3	1	50
6.	Erythromycin	6	60	3	33.3	2	66.7	1	50
7.	Oxacillin	3	30	3	33.3	1	33.3	0	-
8.	Penicillin	3	30	3	33.3	2	66.7	1	50
9.	Vancomycin (MIC)	10	**100**	9	**100**	3	**100**	1	50

All GPC showed 100% sensitivity for Vancomycin except Enterococcus faecalis.

Table 11 : Antimicrobial Susceptibility of Gram Negative Bacteria Causing BSI in IMCU patients

Antibiotics	Pseudomonas aeruginosa (n=11)		Escherichia coli (n=10)		Klebsiella pneumonia (n=10)		Proteus mirabilis (n=1)		Citrobacterkoseri (n=1)		Acinetobacter spp (n=2)	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Ciprofloxacin	7	63.6	6	60	7	70	1	100	0	0	1	50
Cefotaxime	-	-	3	30	2	20	0	0	1	100	-	-
Ceftazidime	5	45.4	3	30	2	20	-	-	-	-	-	-
Ofloxacin	6	54.5	5	50	4	40	1	100	0	0	0	0
Amikacin	6	54.5	9	90	8	80	1	100	0	0	1	50
Gentamycin	5	45.4	9	90	5	50	1	100	0	0	1	50
Cefoperazone-sulbactum	6	54.5	6	60	4	40	0	0	0	0	1	50
Imipenam	9	81.8	10	**100**	10	**100**	1	**100**	1	**100**	2	**100**

All GNB showed high level resistance to third generation cephalosporin. All GNB except Pseudomonas aeruginosa showed 100% sensitivity for Imipenem.

Table 12 :Detection of Methicillin Resistance among Gram positive Cocci

Method	Staphylococcus aureus (n=10)		Staphylococcus epidermidis (n=9)		Staphylococcus schleiferisubsp.schleiferi (n=3)	
	No.	%	No.	%	No.	%
Disc diffusion method	7	70	6	66	2	66.7

7 isolates of staph.aureus (70%), 6 isolates of staph.epidermidis(66%) and 2 isolates staph.schleiferisubsp.schleiferi (66.7%) were found to be MRSA producing organisms.

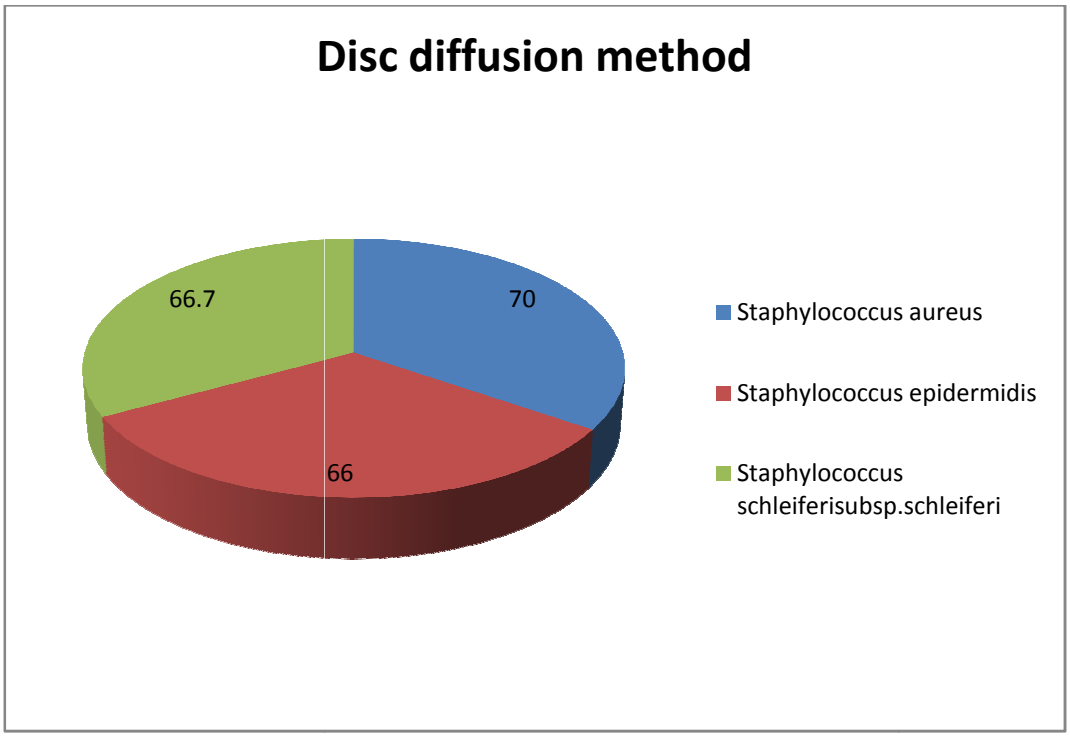


Table 13 : ESBL screening test for gram negative organisms

Organisms	Susceptibility to 3rd Generation cephalosporins		Resistant to 3rd Generation cephalosporins	
	No.	%	No.	%
Klebsiella pneumoniae (n=10)	2	20	8	80
Escherichia coli (n=10)	3	30	7	70
Pseudomonas aeruginosa (n=11)	5	45.4	6	54.5
Acinetobacterspp (n=2)	2	100	0	0

8 isolates of Klebsiella pneumonia, 7 isolates of E.coliand 6 isolates of pseudomonas aeruginosawere found resistant to third generation cephalosporins (3rd GCS).

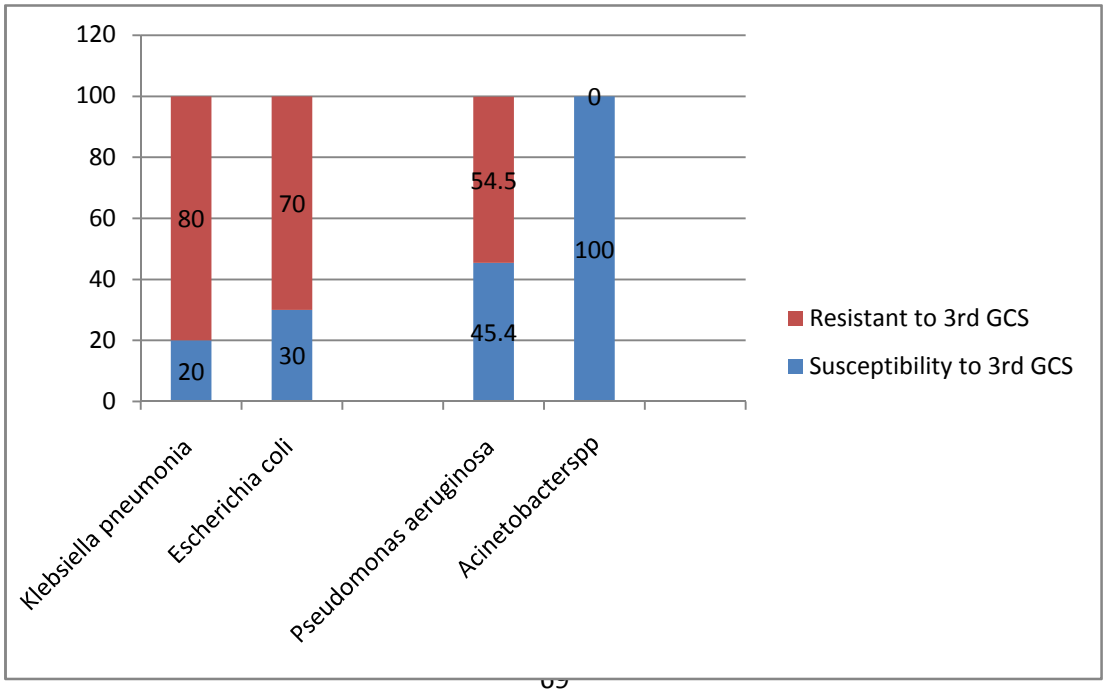


Table 14 : Comparison of Screening tests with DDST & PCDDT

Organisms	No. of screened isolates for ESBL		DDST		PCDDT	
	No.	%	No.	%	No.	%
Klebsiella pneumonia (n=10)	8	80	7	70	7	70
Escherichia coli (n=10)	7	70	6	60	6	60
Pseudomonas aeruginosa (n=11)	6	54.5	6	54.5	6	54.5
Acinetobacterspp (n=2)	2	100	1	50	1	50

23 isolates selected and subjected to confirmatory tests. 20 isolates were confirmed as ESBL producers by PCDDT.

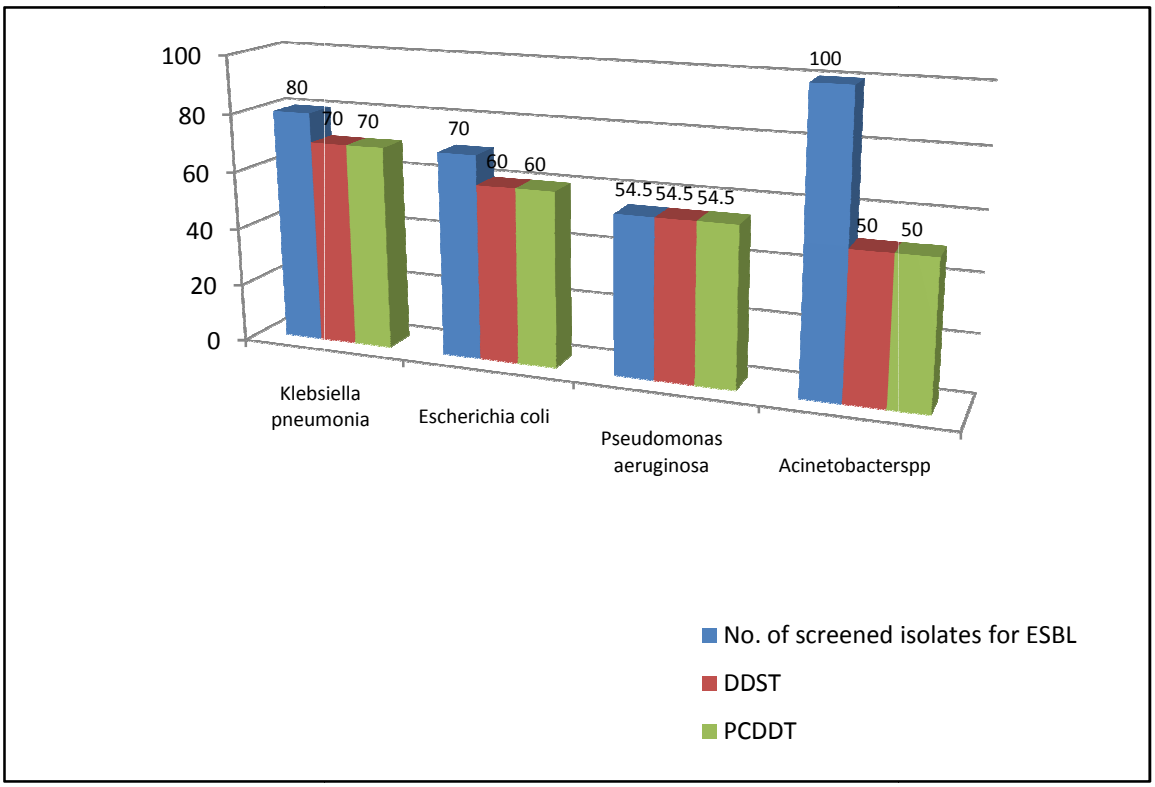


Table 15 : Predisposing Factors of BSI in IMCU Patients

S.No	Predisposing	Cases (n=63)	%
1.	Use of IV Catheter	20	31.74
2.	Use of Urinary Catheter	14	22.22
3.	Mechanical Ventilator	11	17.46
4.	Post Surgical	5	7.93
5.	Peritoneal dialysis / hemodialysis	4	6.34
6.	Chemotherapy on Malignancy	3	4.76
7.	Others	6	9.52

Intravenous catheters are associated with 31.7% and Urinary Catheters are associated with 22.22 % of BSI positive cases.

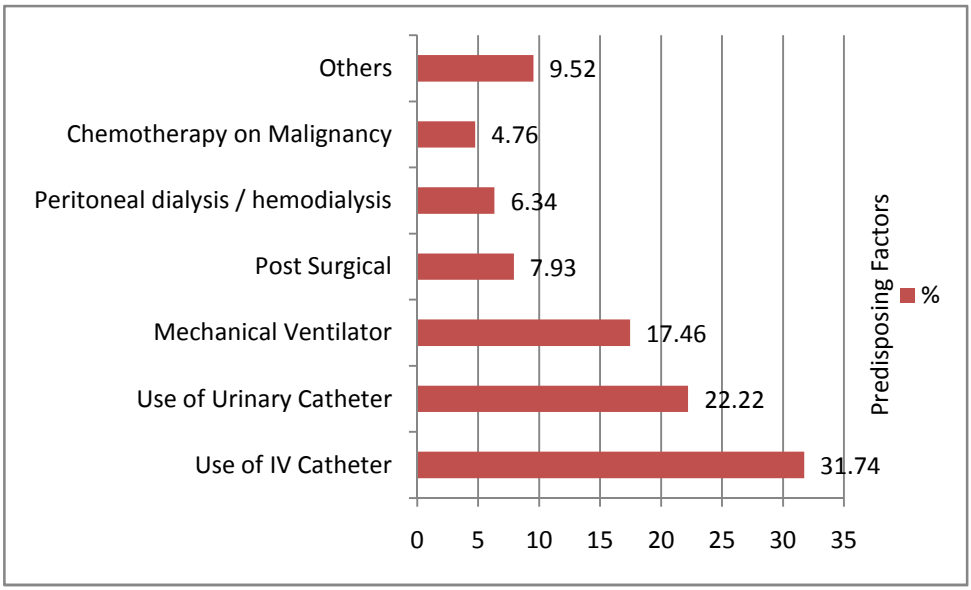


Table 16 :Source of infection in BSI positive cases

S.No	Source	Cases (n=63)	%
1.	IV catheter(CVC)	16	25.4
2.	Urinary tract	14	22.2
3.	Respiratory system	11	17.5
4.	GI tract (Bowel/Peritoneum)	5	7.8
5.	Skin	4	6.3
6.	Biliary tract	1	1.6
7.	Others	8	6.3
8.	Unknown	4	12.7

Intravenous catheters (25.4%) were the common source of infection associated with BSI followed by genitourinary sources (22.2%).

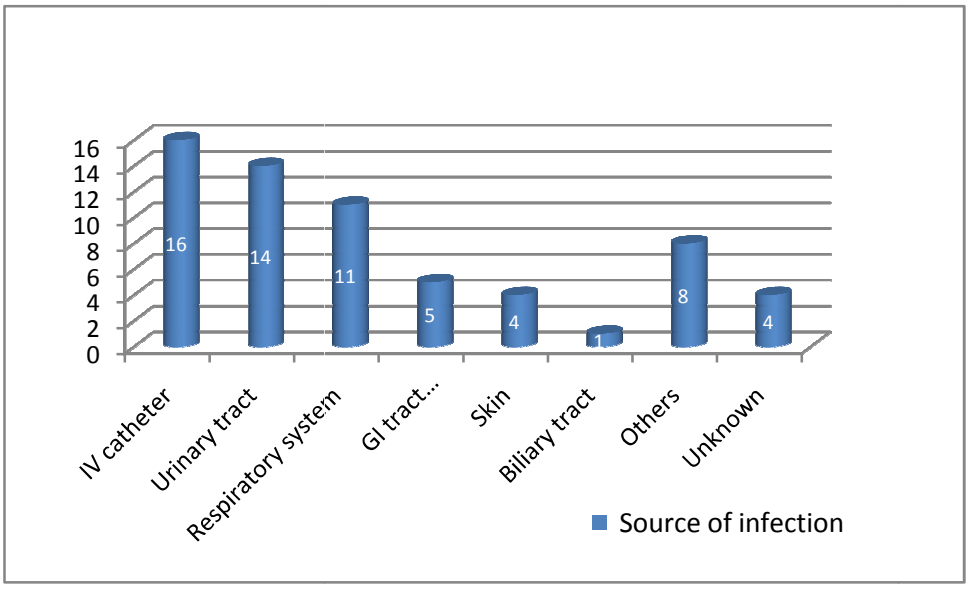


Table 16(a): MINIMUM INHIBITORY CONCENTRATION TEST(MIC) for Vancomycin

S.No.	No. of Isolates	Vancomycin	
		Sensitive (MIC ≤ 2µg/ml)	**Resistant (MIC ≤ 2µg/ml)**
1.	Staph aureus (n=3)	0.5	-
2.	Staph aureus (n=3)	1	-
3.	Staph aureus (n=2)	2	-

All 8 isolates are confirmed as Vancomycin Sensitive.

Table 16(b): MINIMUM INHIBITORY CONCENTRATION TEST(MIC) for GNB by E-Test.

S.No.	Isolate	ESBL Positive $Mix^{+}/Mix \geq 8$		ESBL Negative $Mix^{+}/Mix \leq 8$	
		Ratio	Value	Ratio	Value
1.	E.coli(n=2)	R/0.064	15.625	-	-
2.	Klebsiella Pneumoniae (n=1)	R/0.032	31.25	-	-

Both E.coli and Klebsiella Pneumoniae were ESBL producers.

Table 17 : Antifungal susceptibility tests by Disc diffusion method

	Fluconazole		Itraconazole	
	Sensitive	**Resistant**	**Sensitive**	**Resistant**
C.albicans (n=2)	2	0	2	0
C.tropicalis (n=2)	0	2	0	2

Candida tropicalis was found to be resistant to both fluconazole and Itraconazole.

Table 18 : Antifungal Susceptibility Test by microbroth dilution method

	Amphotericin B		Fluconazole	
	Sensitive (MIC≤1µg/ml)	**Resistant (MIC>1µg/ml)**	**Sensitive (MIC<64µg/ml)**	**Resistant (MIC>64µg/ml)**
C.albicans (n=2)	2 (100%)	0	2(100%)	0
C.tropicalis (n=2)	2 (100%)	0	2(100%)	0

Candida albicans and Candida tropicalis were susceptible to both Amphotericin B and Fluconazole.

Table 19 : Analysis of clinical outcome / mortality of BSI in IMCU

Outcome	Study group (n=150)		BSI positive cases (n=63)	
	No.	%	No.	%
Recovered	111	74	41	65.09
Death	39	26	22	34.92
Total	**150**	**100**	**63**	**100**

35% of mortality rate was observed in BSI positive cases.

Analysis of clinical outcome / mortality of BSI in IMCU

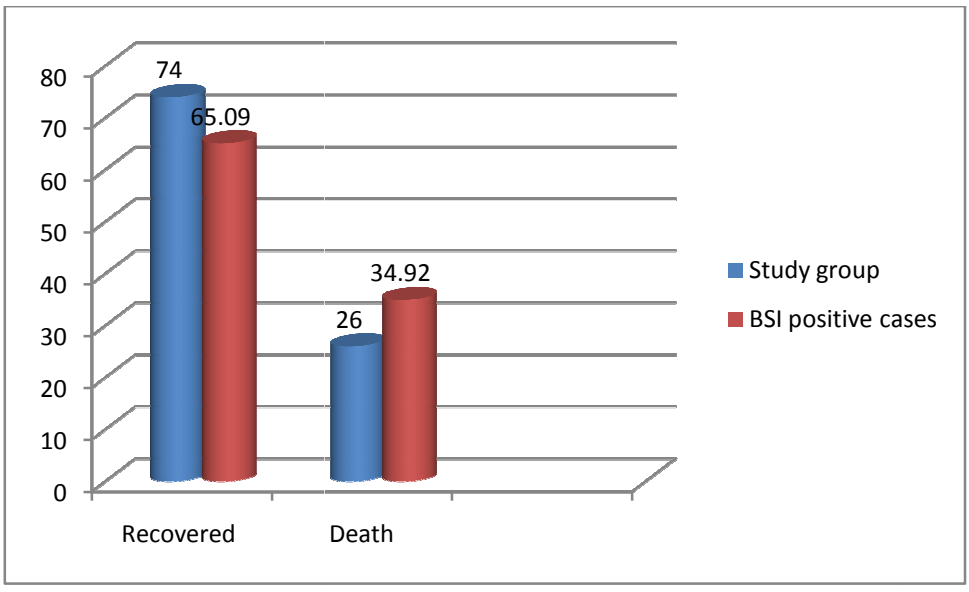
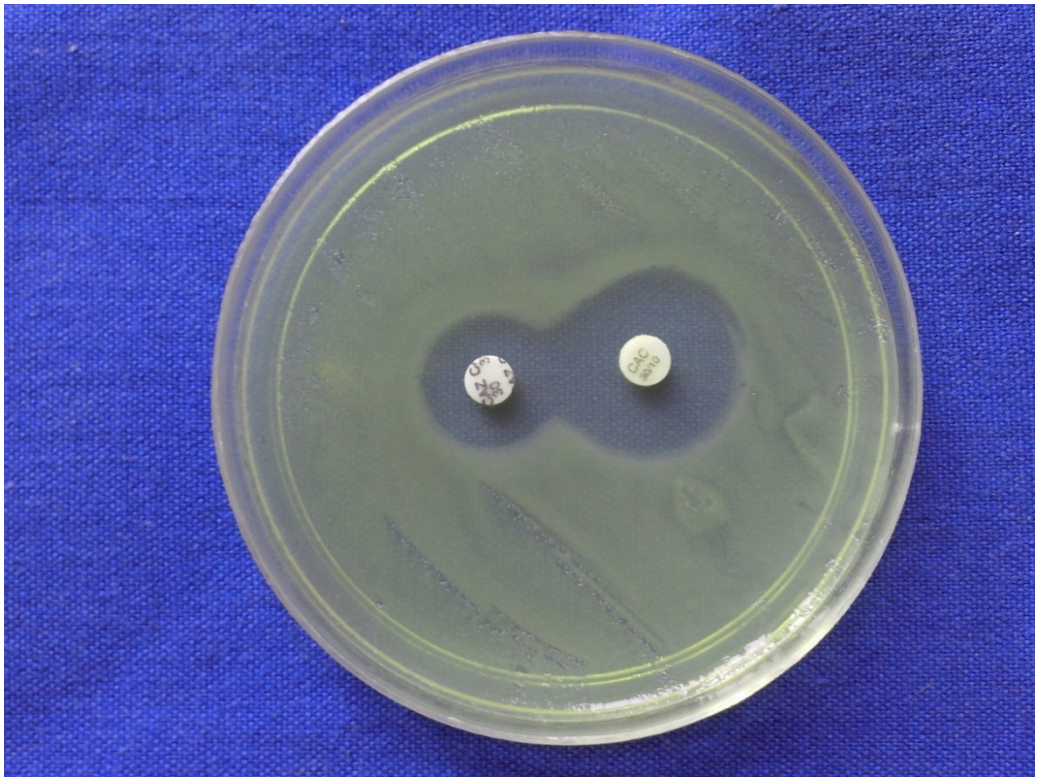


Table 19 : Organism associated with mortality in BSI positive cases of IMCU (n=22)

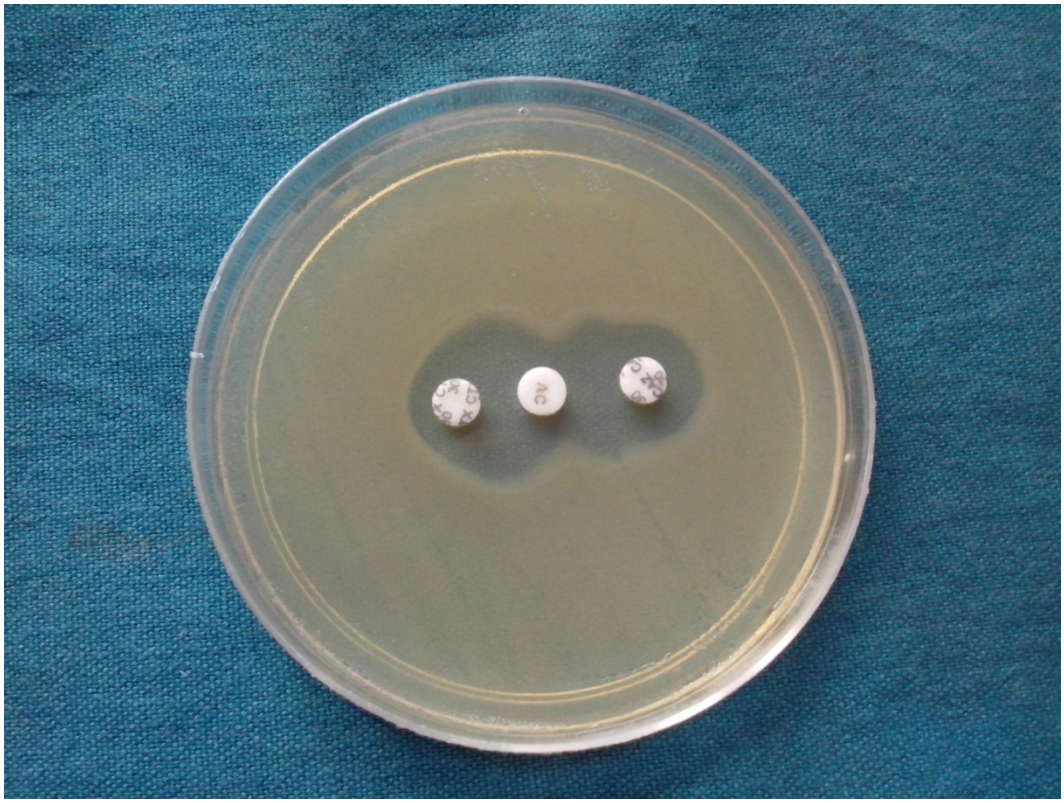
Total No.of deaths	Organism isolated					
	Pseudomonas aeruginosa	**Candida**	**Staph. aureus**	**CONS**	**Klebsiella pneumonia**	**E.coli**
22	8 (31.8%)	3 (13.6%)	5 (22.7%)	3 (13.6%)	2 (9.09%)	1 (4.45%)

In this study total deaths were 22 out of which 8 deaths (31.8%) was due to pseudomonas aeruginosa.

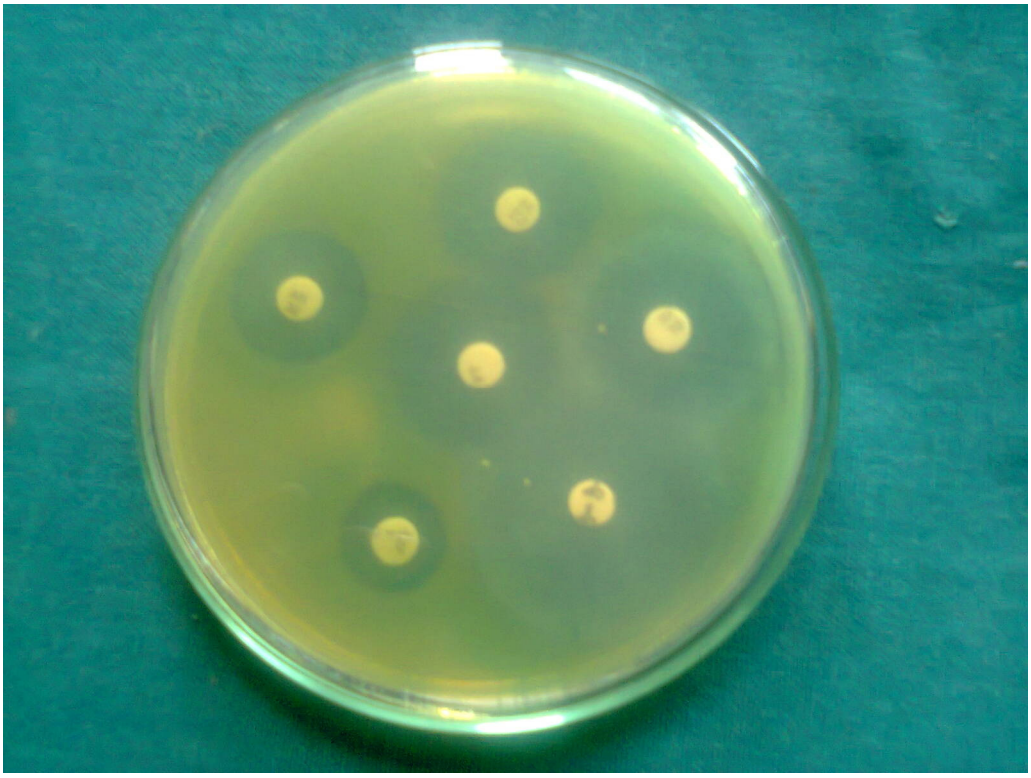
DOUBLE DISK SYNERGY TEST (DDST)



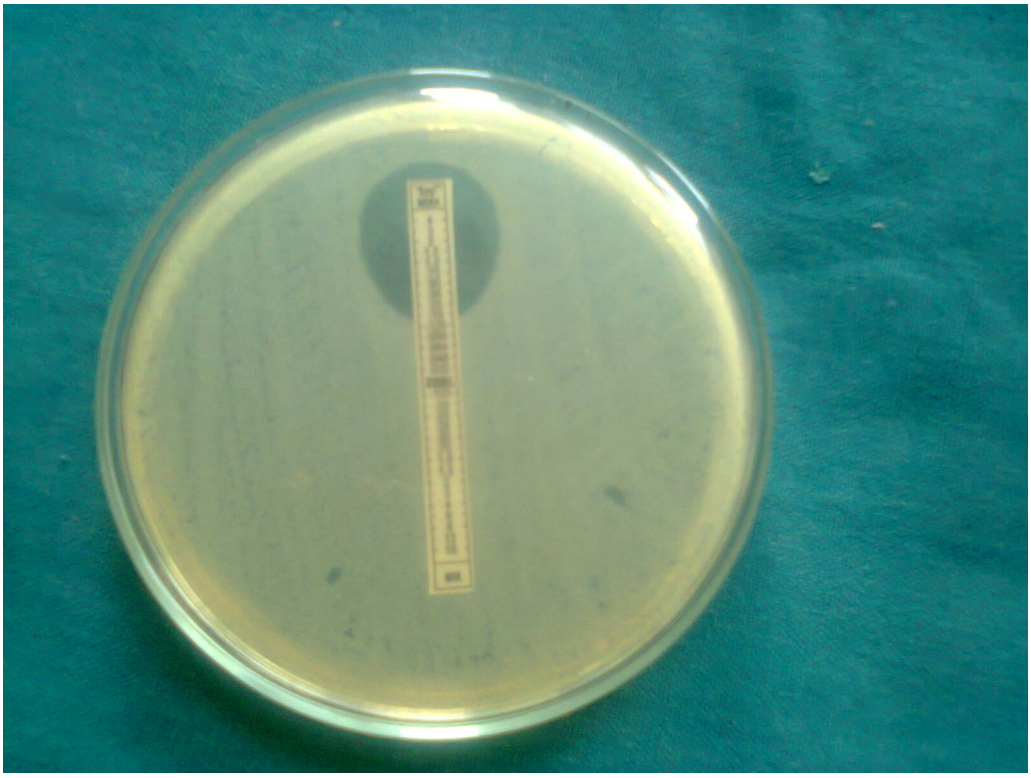
PHENOTYPIC CONFIRMATION DOUBLE DISK TEST(PCDDT)



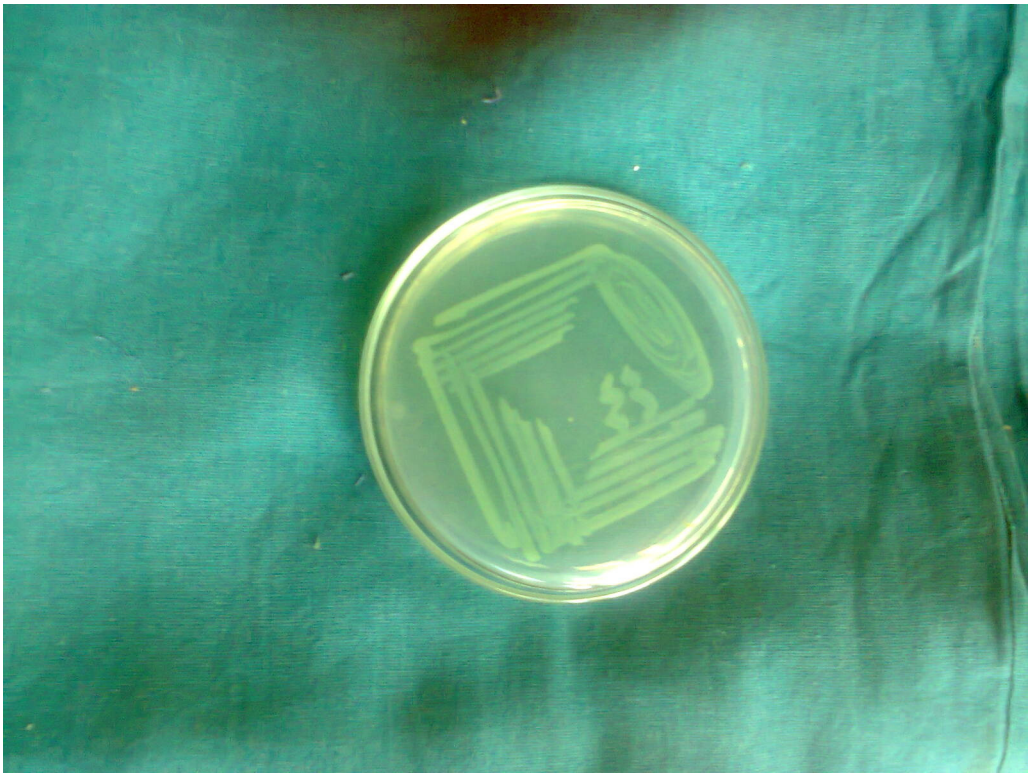
ANTI BIOGRAM – PSEUDOMONAS AERUGINOSA



EPSILOMETER TEST – E.COLI

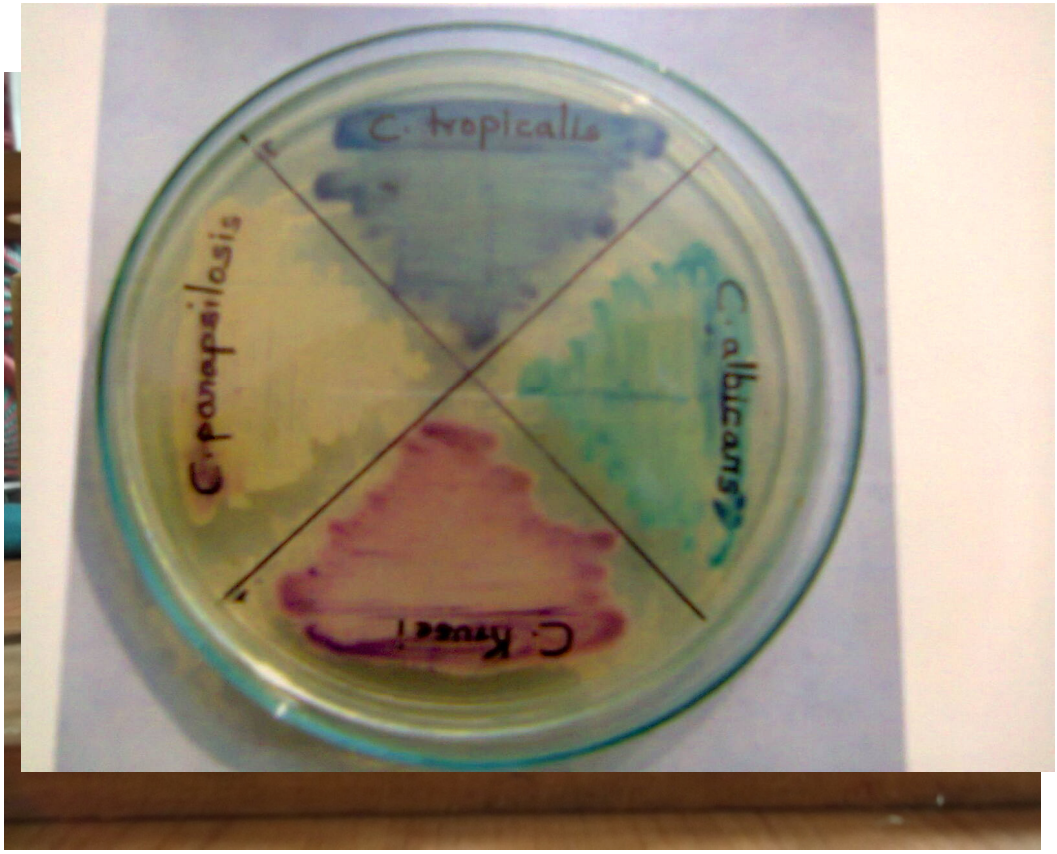


PIGMENTATION OF PSEUDOMONAS AERUGINOSA



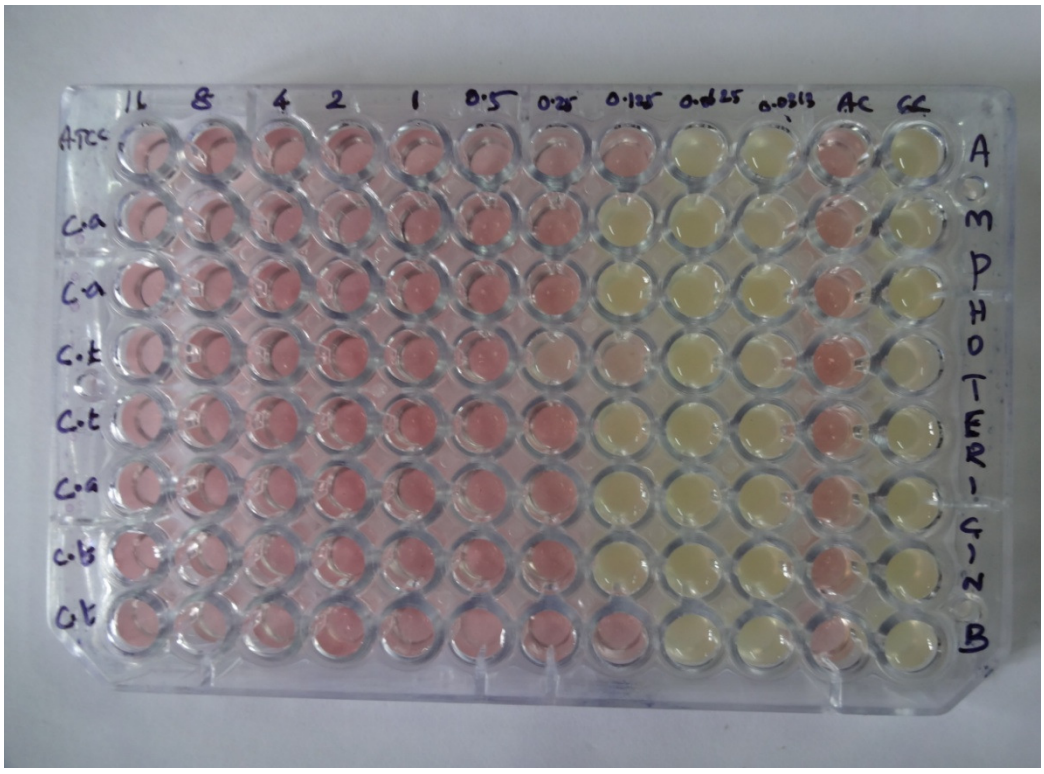
KLEBSIELLA PNEUMONIAE – ON MACCONKEY AGAR

CANDIDA SPECIES ON CHROM AGAR PLATE



MIC OF VANCOMYCIN – MACROBROTH DILUTION METHOD

MIC OF CANDIDA ALBICANS & CANDIDA TROPICALIS



DISCUSSION

Blood stream infections are a major cause of morbidity and mortality among patients in intensive care units (ICUs). The cause of infection in ICU is multifactorial and consequences depends on pathogens associated, source of infection in ICU underlying risk factors, timely intervention and appropriate treatment received. Hence this study was done to determine the bacteremia and fungemia in critical care setup, their source of infection and to determine the antimicrobial susceptibility pattern of the isolates from blood culture.

In the present study, majority of the patients admitted in IMCU with clinical signs of sepsis (under SIRS criteria) were in the age group of 41-50 yrs. This results (Table 2)correlated with the study of **prowle et al,critical care 2007**[43] in which majority of the patients were from 49-73 yrs and the mean age is 61 yrs. In another study done by **Van Gestel et al**[44]**, 2004**, majority of the patients belonged to the age group 64 ± 15 yrs and the male : female ratio is 1:7:1.

There was a male preponderance accounting for 75% in this study (Table3)**. Orsi GB et al**[45] , also reported similar results in which male accounted for 64.8% & females accounted for 35.2% . In another study done by **Derek et al 2001**[46], Males (56.1%) outnumbered females.

Fever was presenting symptom in all 100% of cases in the present study. Since the patients with BSI presenting clinically had other conditions like Urinary symptoms&Respiratory distresswere seen in 10% of cases,tubercular infection in 16% and neurological manifestations in 8% of patients. Anemia (43%) and icterus (10.8%) were consistently present in majority of cases as in (Table 4). .

Co-morbid conditions reported in this study were Respiratory disease (20%), renal disease (12%), Cirrhosis (gastroenterology) in (6%) and the independent risk factors were diabetes mellitus (33 %) and Hypertension (18 %).

In this study, blood stream infections were found to be positive in 63 of 150 cases. (Table 5). This correlated with the study of **Jordivalles et al,2009**[47], in which the prevalence rate of blood stream infection was 30 - 40% of cases.

Similar study by **Pittet et al 2009**[48]**&Rello et al 2009**[48], also gave the prevalence of Bacteremia was 30%. Epidemiology of infection among culture positive BSI cases was shown in IMCU patients(Table 5) community acquired and nosocomial bacteremia were found to be 28 % &55 % respectively. 95% of the infection were monomicrobial as revealed by **Garrouste Orgaset al**[49] **2009** in their study. A recent nationwide surveillance study conducted in United state hospital (scope) reported

that51% of Hospital acquired BSI occurred in ICUS.In another study by **Stephane et al 2004**[60], nosocomial acquired BSI (60%) is more common than community acquired BSI (40%) and this correlated with this study.

Etiological agents were more commonly Gram negative bacteremia (55.55%) with Pseudomonas aeruginosa being the most common isolate. Gram negative bacteremia (55%) is greater than gram positive bacteremia (35%).Gram negative bacteria isolated from the BSI culture positive cases include E.coli (15.87%), Klebsiellapneumoniae (15.87%),Acinetobacterspp (3.17%), Proteus mirabilis (1.58%), Citrobacterkoseri (1.58%). Gram positive bacteria accounted for 38.09 % of the isolates. Among gram positive bacteria coagulase negative Staphylococcus (CONS) was isolated in most samples (19%) than staphylococcus aureus (15%). Among CONS, Staph epidermidis accounted for 14.28% and Staphylococcus schleiferi subspp. schleiferi accounted for 4.76% of total isolates. Fungal isolates isolated were candida albicans 3.17% & candida tropicalis 3.17%.

Jamal et al[51]**2009** found that most common isolate in BSI was Coagulase negative staphylococci (46%). In a similar study done by **Rello et al &Valles et al 2009**, CONS accounted for 49.8% and Pseudomonas aeruginosa accounted for 32.6 % of the BSI which correlated well with this study. Similarly **Ogstonet al**[52]**2009** reported coagulase negative staphytococcus to be the most common isolate.In a study conducted in

Brazilian hospital ICU & Staphylococcus aureus was found to be common isolate which was in contrast to our study.

In the present study, aerobic gram positive cocci were isolated in 38.09%, aerobic gram negative bacilli in 55.55%. This correlates with the study done by **S Bhattacharya et al[53],** 2002 where GNB accounted for 56.2 %& GPC accounted for 24%.

From 63 BSI positive cases CONS was the most common pathogen isolated in 12 (19.8%) followed by Pseudomonas aeruginosa (17.55%).In coagulase negative staphylococci, S.epidermidis was isolated in (14.28%) of cases and S schleiferi subspp. Schleiferi was isolated in (4.76%) of cases. E.coli was isolated in (15.87%), Klebsiella in (15.87%), Acinetobacter in (3.17%), Proteus mirabilis in (1.58%), Citrobacter koseri in(1.58 %) of cases. Staphlococcus aureus was present in 38.09% of cases and Enterococcus faecalis in 3.17% of BSI positive cases

Gram positive bacteria showed 100% sensitivity to Vancomycin. All Enterobacteriaceae showed 100% sensitivity to Imipenem.

The commonest bacteria isolated in this study, CONS showed 100% sensitivity to Vancomycin, 80% sensitivity to Amikacin, 77% to ciprofloxacin 60% sensitivity to Erythromycin.

The second common isolate among GPC was S.aureus which showed 100% sensitivity to Vancomycin, 80%to Amikacin, 60% to Erythromycin, 50% to ciprofloxacin.

70% of Staph aureus and66% of Staph epidermidis were found to be Methicillin resistant. This indicates MRSA and MRS epidermidis was more common in ICU pts. This indicates the use of inadequate antibiotics during empirical therapy, invasive procedures like IV catheters which breach the host defense mechanism and longer duration of hospitalization may selectively enhance the growth of drug resistant pathogens.

Sensitivity to Vancomycin was detected by Macrobroth dilution method. All isolates showed MIC within sensitivity range (<2µg/ml).

In spite of this the septicaemic patients have high mortality rate due to invasive procedures (ie health care interventions) leading to biofilm formation and length of stay in hospital and associated co-morbid conditions.

Enterococcus faecalis showed 50% sensitivity to Vancomycin, 50% to ciprofloxacin and Amikacin. Isolate identified as Vancomycin resistant Enterococci (VRE) showed MIC >32µg/ml by macrobroth technique.

Among gram negative organisms Pseudomonas aeruginosa was the most common isolate which showed 81%Imipenemsensitivity , 61% to

Ciprofloxacin, 55% to CFS , 55% to Amikacin, 45% to Gentamycin, 45% to ceftazidime.

Among the mechanism of resistance to third generation cephalosporins, production of ESBL is the most common mechanism observed in this study.18.19% of Pseudomonas aeruginosa were found to MBL producers. (Table 10). However no isolate was found to be AmpC producers.

Out of 11 isolates of Pseudomonas aeruginosa screened for ESBL and MBL production 6 (54.5 %) were found to be ESBL positive and 2 (18.2%) were found to be MBL positive respectively by screening method and confirmatory tests (Table 13).

Similarly ESBL producers were found most commonly in Klebsiellapneumoniae (70%), E.coli (60%), Pseudomonas aeruginosa (54%) and Acinetobacterspp (50%).

The common source of blood stream infection in IMCU patients include the lungs, skin, subcutaneous tissue and intravascular catheters. In this study, the most common source of BSI was IV catheters (25.4%), followed bygenitourinary sources (22.2%). This shows that common source ofBSI was Intravascular catheters(mainly central venous catheter in thus study) which is an important predisposing factor for Bacteremia.This

correlated with the study of **prowle et al 2004**. No intravenous drug abusers were found in this study.

Candida albicans was reported in 2 (3.17%) of cases and Candida tropicalis 2 (3.17%) of cases in the present study. This is similar to **Lymn et al**[55] who reported, C.albicans & C.tropicalis (24%) of equal incidence forBSI in ICU patients.**Grace et al , Prasad et al ,Baradkar et al**, **pfeller et al** reported C. albicans (60%) to be the most common isolate from BSI in critical care units followed by Candida tropicalis (30%). **Capoor et al** [54] reported Candida tropicalis as the causative agent for 38.7% of candidemia.

Both Candida albicans and Candida tropicalis showed 100% sensitivity to Amphotericin B. Fluconazole resistance was observed in C.tropicalis isolate. A study conducted by **Law D et al** reported that C.tropicalis (20 %) were resistant to Fluconazole.

There was a total of 22 death in BSI positive cases in this present study. The overall mortality rate is 35%. Prowle et al 2009 reported over all mortality rate in ICU about 40%. Out of 22 deaths, 31.8% were due to Pseudomonas aeruginosa. Out of 4 candidal spp.3 deaths occurred in IMCU patients.This shows high mortality rate for Fungemia in critical care set up. Among deaths due to Bacteria, Pseudomonas spp had high mortality rate due to invasive procedures, long hospital stay& Biofilm formation in IMCU patients.

SUMMARY & CONCLUSION

A prospective study was undertaken over a period of one year in 150 patients. To determine the bacterial and fungal isolates causing septicaemia in patients admitted to IMCU (Intensive Medical Care Unit).

Fever (10%) and Respiratory distress (19.45%) were the most common presenting symptoms.

The culture positivity is 42%. The Spectrum of bacterial isolates were 38.09% GPC (Gram Positive cocci), 55.55% GNB (Gram Negative Bacilli). Fungi contributed to remaining 6.34%

The common Organisms isolated were CONS (Coagulase negative staphylococci) 19.04% and Pseudomonas aeruginosa 17.46%.

The Epidemology of BSI in IMCU in the study was nosocomial acquired contributing to 55.6% of cases.

All GPC showed 100% sensitivity for Vancomycin except Enterococcus faecilis, 80% sensitivity to Amikacin, 77% sensitivity to Ciprofloxacin and 60% sensitivity to Erythromycin.

All GNB except Pseudomonas aeruginosa showed 100% for Imipenam, 90% sensitivity to gentamycin 80% sensitivity to Amikacin, 70% sensitivity to Ciprofloxacin and 60% sensitivity to Erythromycin.

Among the Staphylococcus aureus 70% were methicillin resistant and 30% were methicillin sensitive. Staphylococcus epidermidis 66% were methicillin resistant and Staphylococcus schleiferi subsp.schleiferi 66.7% .were methicillin resistant.

70% of Klebsiella pneumoniae & 60% of E.coli were ESBL producers.

Candida was found in 6.34% of Septicaemic cases. The commonest being C.albicans (3.16%) & C.tropicalis (3.16%).

All Candida isolates were sensitive to Amphotericin B.

In this study IV Catheters are main predisposing factor for BSI in IMCU contributing to 31.7% of cases.

The most common source of infections are invasive procedures like Central Venus Catheterization (CVC) in critical care setting followed by genitourinary sources in Hospital acquired blood stream infections.

Presence of Methicilin resistant Staphylococcus aureus(MRSA) and Extended spectrum β lactamase (ESBL) producing gram negative bacilli in IMCU setting leading to grave prognosis. So irrational use of antibiotics leads to emergence of resistant organisms.

To reduce the morbidity and mortality in IMCU setting following interventions are followed.

1. Adherence to guidelines on insertion and care of Central Venous Catheterization.
2. Use of antimicrobial or antiseptic impregnated Catheters
3. Improved hand hygiene.

PROFORMA

IP No:

- Name :
- Age :
- Sex :
- Occupation :
- Address :
- Presenting complaints :
- Risk factors / demographic profile of the parties

Underlying illness

Diabetes mellitus

Chronic Lung disease

Chronic Heart failure

Chronic Renal failure

Chornic Liver disease

Uremia

Neoplasm

Trauma

Coma

Pronged stay in hospital

Prolonged stay in ICU

Broad spectrum antibiotic & its duration

Immunosuppressive therapy

Chemotherapy

HIV/AIDS

Arteriovenous graft

Splenectomy

Procedures

Intra venous Catherization

Reason & duration of catheterization

Any other invasive procedure

Prior Surgery

Hospital care personnels

 Physical examination
 Laboratory evaluation

Biochemical

Blood sugar

HP Alc

Urea

Creatinine

Neutropernia

Other investigation

WBC count

ESR

USG

Microbiological investigation:

- Gram's Stain:
- Culture :

  BHI Broth

  Fungal culture on SDA

Antimicrobial susceptibility pattern

AntiBacterial susceptibility by Disc diffusion

Antifungal susceptibility by Microboth dilution technique.

INSTITUTIONAL ETHICAL COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No: 04425305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. N. Deepa
PG in MD Microbiology
Madras Medical College, Chennai -3.

Dear Dr. N. Deepa

The Institutional Ethical Committee of Madras Medical College reviewed and discussed your application for approval of the project / proposal / clinical trail entitled " A study on the microbiological profile of blood stream infections in patients admitted in intensive medical care unit in a tertiary care hospital" No 45082010.

The following members of Ethical committee were present in the meeting held on 24.08.2010 conducted at Madras Medical College,Chennai -3.

1. Prof. S.K. Rajan, MD	-- Chairperson
2. Prof. J. Mohanasundaram, MD,Ph.D,DNB Dean, Madras Medical College, Chennai -3	-- Deputy Chairman
3. Prof. A. Sundaram, MD Vice Principal , MMC, Chennai -3	-- Member Secretary
4. Prof R. Nandhini, MD Director, Institute of Pharmacology, MMC, Ch-3	-- Member
5. Prof. C. Rajendiran , MD Director, Institute of Internal Medicine, MMC, Ch-3	-- Member -- Member
6. Prof. Md. Ali, MD, DM Professor & Head ,,Dept. of MGE, MMC, Ch-3	-- Member -- Member
7 Prof. Shantha Ravishankar, MD Professor of Neuro Pathology, MMC, Ch-3	-- Member
8. Tmt. Arnold Soulina	-- Social Scientist

We approve the trail to be conducted in its presented form.

Sd /. Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report

Member Secretary, Ethics Committee

APPENDIX

STAINING

I) Gram staining

Methyl violet (2%)- 10g methyl violet in 100 ml absolute alcohol in 1 litre of distilled water

Grams iodine – 10 g Iodine in 20g KI (fixative)

Acetone- Decolourising agent

Carbol fuchsin 1%- secondary stain

2) CULTURE MEDIA

Brain Heart Infusion Biphasic medium

Sodium citrate 1gm

Sodium chloride 4 gm

Sodium phosphate 5 gm

Dextrose 10 gm

Peptone 10 gm

Brain heart Infusion Broth

Brain infusion broth 250 ml

Heart infusion broth 750 ml

Sodium polyanethol sulponate 0.25gm

Obtain Ox brain and heart. Remove all fat from the heart.cut into small pieces and grind . add distilled water three times and

keep at 4^0 C overnight. From the brain , remove meninges fully and then , weigh. Add distilled water and mash by using hand keep in the cooler over night. Next morning boil the brain and heart separately for 30 minutes. Then filtered through cotton layer. Measure each broth separately. Mix both infusions and the remaining ingredients. dissolve well and adjust ph of the entire amount to 7.4 to 7.6 Autoclave at 121 c for 15 minutes.

Brain heart infusion agar slant

Same as above , in addition it contain agar. Autoclave at 121^0 c for 15 minutes. Distribute in screw capped bottles and slant is allowed to solidify, then broth is distributed in bottles.

Trypticase soy broth

Pancreatic digest or casein 17.0 gram

Enzymatic soy Digest 3.0 gram

Sodium chloride 5.0 gram

Dipotassium phosphate 2.5 gram

Dextrose 2.5 gram

Sodium polyanethol sulfonate 0.3 gram

Final ph adjusted to 7.3 ±0.2 . autoclave at 121^0 C for 15 minutes and distribute in screw capped bottles .

Blood Agar (5% sheep blood agar)

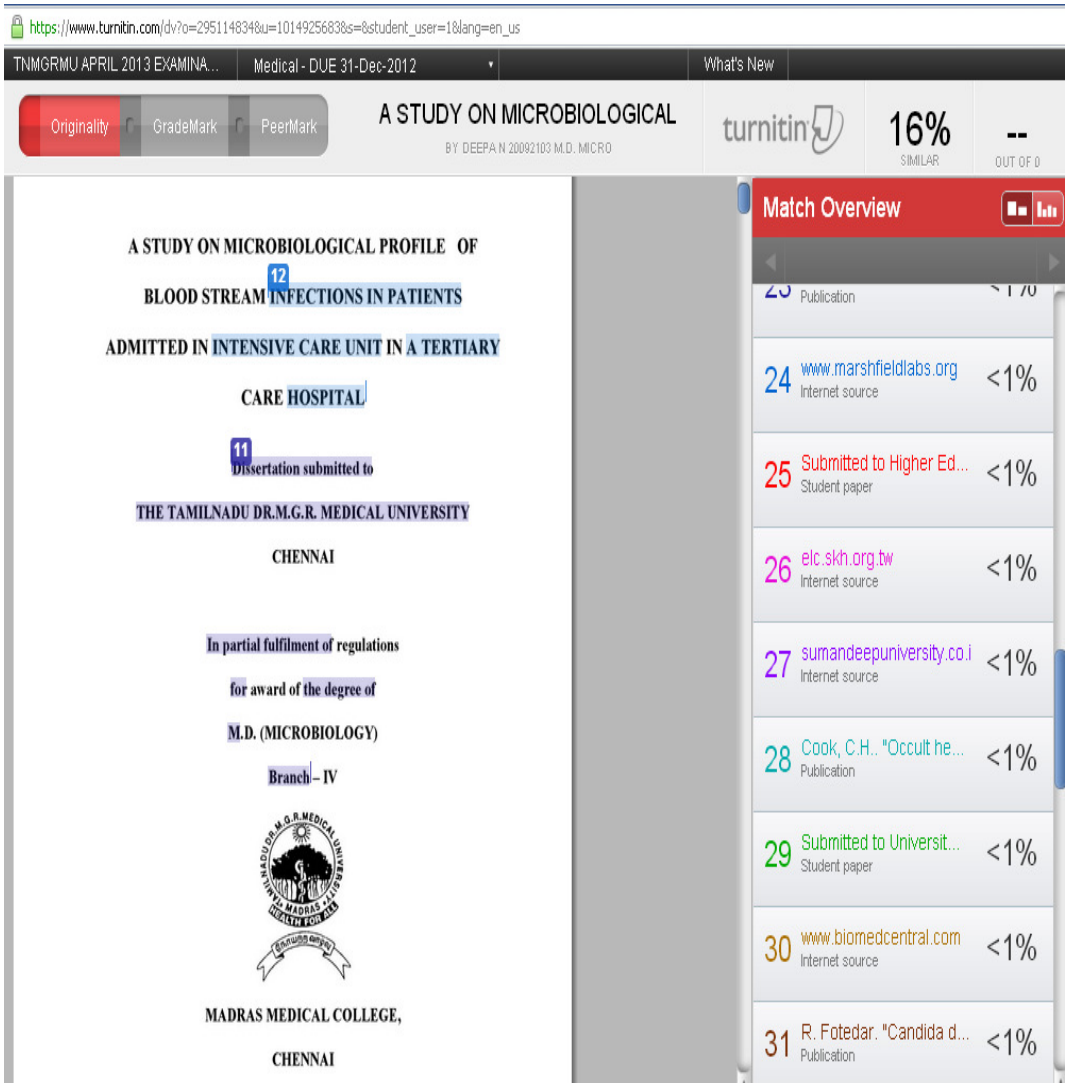
Peptone	- 10g
Nacl	- 5g
Distilled water	-1 Ltr
Agar	- 10g

Dissolve ingredients in distilled water by boiling , and add 5% sheep blood (sterile) at 55^0 C adjust ph to7.4 .

Cornmeal agar

Cornmeal	-40g
Agar	-15g
Water	-1 liter

Boil the corn meal in 1 liter of water for 60 min . filter through muslin and add the agar . Steam to dissolve, dispense in required amounts and autoclave at 115^0 C for 30 min. allow to cool to 50^0 C and pour approximately 20 ml amount into petri dishes.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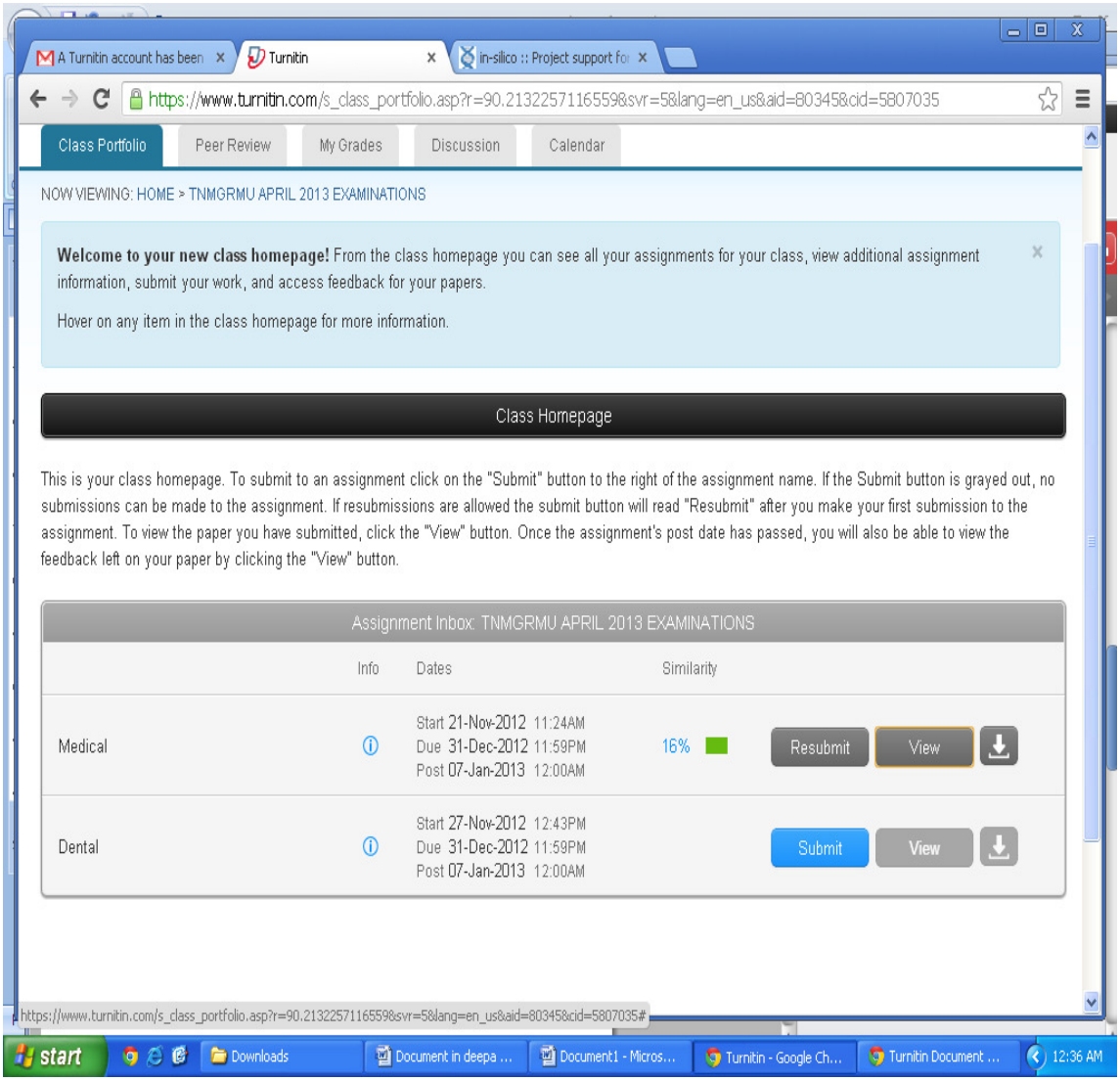
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ABBREVIATIONS

BSI – BLOOD STREAM INFECTIONS

CD – CLUSTER DIFFERENTIATION

ESBL- EXTENDED SPECTRUM BETA LACTAMASE

MRSA-METHICILLIN RESISTAN STAPHYLOCOCCUS AUREUS

CONS-COAGULASE NEGATIVE STAPHYLOCOCCUS AUREUS

GPC-GRAM POSITIVE COCCI

GNB-GRAM NEGATIVE BACILLI

s.no	NAME	AGE	SEX	FEVER	HR>90/MIN	RR>20/MIN	ASCITES	ANAEMIA	ICTERUS	LYMPHADENOPATH	NEUROLOGICAL SYMPTOMS	URINARY SYMPTOMS	SKIN LESION	TUBERCULAR INFECTION	CVC	MECHANICAL VENTILATOR	URINARY CATHETER	CHEMOTHERAPY	HAEMODIALYSIS	POSTSURGICAL	RESP SYSTM
1	VENKATACHALAM	23	M	Y				Y			Y				Y	Y	Y				
2	KASI	70	M	Y					Y			Y			Y						
3	SIVASUNDAR	38	M	Y				Y								Y	Y				
4	SINDHU	13	F	Y	Y			Y		Y										Y	
5	ARUNAGIRI	57	M	Y		Y						Y	Y		Y	Y					Y
6	RAJAMMA	55	F	Y	Y	Y			Y	Y				Y			Y				Y
7	murugan	32	M	Y				Y										Y			
8	MANNAN	16	M	Y	Y														Y		
9	KARTHIKRAJA	17	M	Y	Y				Y	Y									Y	Y	
10	ROJA	16	F	Y				Y						Y							
11	KALIDOSS	36	M	y				Y									Y				
12	PURUSHOTHAMAN	50	M	Y				Y													
13	SETHURAMAN	66	M	Y	Y	Y		Y		Y		Y	Y	Y							
14	SIVA	20	M	Y					Y	Y					Y					Y	
15	MUNUSAMY	65	M	Y	Y	Y				Y			Y				Y				Y
16	BABU	23	M	Y	Y			Y		Y											Y
17	IBRAHIM	40	M	Y	Y			Y				Y				Y		Y	Y		
18	MANOHARAN	23	M	Y																	
19	MANIKANDAN	25	M	Y	Y				Y												
20	DILLIBABU	28	M	Y				Y									Y				
21	CHINNAIAH	22	M	Y	Y						Y					Y					
22	RAJA	23	M	Y				Y				Y				Y					
23	BALACHANDAR	16	M	Y	Y				Y	Y											
24	MURUGAN	40	M	Y	Y										Y						
25	JAGADEESWARI	34	F	Y			Y									Y	Y				
26	SIVA	20	M	Y	Y				Y	Y	Y										
27	MARI	48	M	Y	Y	Y		Y		Y			Y	Y							Y
28	DHANASEKAR	55	M	Y	Y		Y							Y	Y	Y		Y			
29	ASHIYA BEGUM	13	F	Y				Y		Y	Y	Y									
30	MURUGAN	22	M	Y	Y				Y						Y						
31	KARTHICK	17	M	Y	Y	Y	Y														Y
32	ANANDRAO	27	M	Y	Y					Y	Y	Y				Y					
33	SAROJA	50	F	Y	Y		Y					Y									
34	SEKAR	36	M	Y		Y		Y													Y
35	PRAVEEN	13	M	Y	Y				Y	Y							Y				
36	SIVA	42	M	Y	Y			Y					Y	Y		Y					
37	CHAKKARAVARTHI	40	M	Y	Y	Y		Y		Y											Y
38	SENGOLMANI	30	M	Y	Y		Y									Y		Y			
39	LOGANATHAN	22	M	Y					Y											Y	
40	ELUMALAI	28	M	Y	Y			Y			Y				Y						
41	MOHAN	34	M	Y				Y								Y					
42	RAVI	42	M	Y	Y			Y													
43	KUPPAN	55	M	Y				Y			Y		Y	Y			Y				

44	MARKANDAN	38	M	Y						Y					Y	Y					
45	RAMAN	65	M	Y	Y		Y				Y										
46	SUMATHY	27	F	Y	Y	Y											Y			Y	Y
47	HAJIRA	28	F	Y			Y										Y	Y			
48	SHAKIR	15	M	Y	Y			Y				Y									
49	MANIVANNAN	21	M	Y	Y	Y												Y			Y
50	MANIVEL	58	M	Y	Y											Y			Y		
51	mari	32	M	Y				Y													
52	RAJESHKANNA	30	M	Y	Y		Y									Y					
53	RAVI	47	M	Y	Y			Y					Y	Y							
54	PANDURANGAN	38	M	Y	Y						Y	Y				Y					
55	GIRI	36	M	Y				Y													
56	RAMAN	45	M	Y	Y		Y					Y	Y								
57	RANJITH	18	M	Y	Y																
58	KARUNAKARAN	27	M	Y	Y			Y													
59	ARMANALLA	50	M	Y				Y													
60	MAASILAMANI	40	M	Y	Y																
61	MANIKANDAN	17	M	Y	Y	Y							Y	Y							Y
62	ARMANALLA	50	M	Y	Y			Y												Y	
63	ANDIASAMY	67	M	Y	Y			Y					Y	Y							
64	MURUGAN	41	M	Y	Y	Y											Y				Y
65	SHEELA	23	F	Y																	
66	SHAKIR	38	M	Y																	
67	KUSHRAJ	33	M	Y													Y				
68	VENKATESAN	36	M	Y					Y												
69	KANNAN	47	M	Y																	
70	SARATHKUMAR	18	M	Y	Y			Y			Y	Y									
71	GOVINDASAMY	54	M	Y		Y			Y										Y		Y
72	VENDA	25	F	Y	Y			Y										Y			
73	BHARGAR	25	M	Y			Y														
74	KRISHNAVENI	52	F	Y	Y			Y						Y							
75	AKASHRAJ	12	M	Y																	
76	SAUKATH ALI	38	M	Y	Y	Y												Y			Y
77	UMACHANDRAN	39	M	Y				Y										Y			
78	KARTHIKEYAN	39	M	Y	Y			Y													
79	GUNASEKAR	45	M	Y																Y	
80	DEVI	32	F	Y	Y																
81	SUNITHA	31	F	Y	Y			Y			Y	Y							Y		
82	KOTHANDAN	50	M	Y	Y			Y								Y					
83	PREMKUMAR	13	M	Y	Y						Y										
84	VENKATESAN	40	M	Y	Y	Y			Y												Y
85	GUNASEKARAN	45	M	Y				Y								Y					
86	SAVITHA	55	F	Y	Y			Y		Y											
87	RAJA	55	M	Y	Y	Y		Y													Y
88	MANI	50	M	Y			Y									Y					
89	SUDHA	31	F	Y																	
90	MURUGAN	38	M	Y																	

91	JEBA	45	F	Y				Y						Y								
92	GOPI	35	M	Y	Y			Y				Y										
93	RAJA	28	M	Y	Y		Y															
94	SURYA	29	F	Y	Y			Y					Y	Y								
95	RAMESH	46	M	Y			Y															
96	PREMKUMAR	24	M	Y	Y		Y					Y										
97	SAVITHA	18	F	Y	Y												Y					
98	SURESH	38	M	Y	Y			Y							Y							
99	SENTHIL	35	M	Y	Y				Y						Y	Y						
100	BABU	29	M	Y					Y					Y	Y							
101	RAJU	18	M	Y	Y		Y															
102	RAMU	19	M	Y				Y														
103	RAMESH	31	M	Y	Y			Y													Y	
104	SAROJA	15	F	Y	Y																	
105	LEELA	23	F	Y				Y		Y												
106	ASHOK	29	M	Y				Y														
107	PALANI	32	M	Y	Y				Y						Y		Y					
108	RAMAIAH	26	M	Y	Y			Y														
109	VALLI	40	F	Y	Y								Y	Y								
110	MUNUSAMY	52	M	Y	Y								Y	Y			Y					
111	MURUGAN	38	M	Y				Y														
112	NARAYANAN	55	M	Y	Y			Y					Y	Y								
113	MANI	44	M	Y				Y							Y	Y						
114	RANJITH	48	M	Y	Y			Y				Y	Y	Y								
115	SANGITA	24	F	Y	Y												Y					
116	SUKUMAR	41	M	Y																		
117	BABU	45	M	Y				Y		Y					Y							
118	GIRI	36	M	Y				Y														
119	KRISHNAN	24	M	Y	Y																	
120	PRABHU	37	M	Y				Y														
121	BANU	33	F	Y										Y	Y							
122	BALA	50	M	Y												Y						
123	SATHISH	43	M	Y																		
124	INDRAN	42	M	Y											Y		Y					Y
125	RAJINI	48	M	Y	Y																	
126	SARALA	24	F	Y	Y			Y														
127	SASIKUMAR	36	M	Y											Y							
128	KUMAR	43	M	Y				Y														
129	MARI	36	M	Y				Y						Y			Y					
130	RAVI	26	M	Y	Y											Y				Y		
131	PRAKASH	37	M	Y				Y							Y							
132	MALA	30	F	Y			Y							Y			Y					
133	KATHIR	45	M	Y		Y																

134	SUGUNA	40	M	Y										Y	Y				Y		
135	RAMU	48	M	Y	Y																
136	PARVATHI	24	F	Y	Y			Y						Y			Y				
137	VIGNESH	44	M	Y													Y				
138	BALAKUMAR	45	M	Y				Y						Y	Y						
139	MANI	36	M	Y				Y													
140	KUMAR	24	M	Y	Y																
141	BALA	37	M	Y											Y						Y
142	TAMILSELVI	32	F	Y													Y				
143	VARUN	32	M	Y												Y					
144	RAMACHANDAR	48	M	Y	Y			Y													
145	ROHINI	42	F	Y	Y			Y							Y						
146	MARAN	34	M	Y																	
147	STALIN	45	M	Y				Y						Y		Y					
148	KUMAR	36	M	Y				Y							Y		Y				
149	KAMAL	33	M	Y	Y																
150	KARTHIK	37	M	Y		Y															